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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 857 788 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
12.08.1998 Bulletin 1998/33

(51) Int Cl.[6]: **C12N 15/55**, C12N 9/16, C12P 19/38

(21) Application number: **97309365.1**

(22) Date of filing: **20.11.1997**

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **21.11.1996 JP 311103/96**
18.06.1997 JP 161674/97

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Remarks:
The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Method for producing nucleoside-5'-phosphate ester

(57) A method for producing nucleoside-5'-phosphate ester involves phosphorylating nucleoside biochemically.

Nucleoside-5'-phosphate ester is produced by allowing an acid phosphatase, especially an acid phosphatase having an increased affinity for a nucleoside and/or an increased temperature stability, to act at pH 3.0 to 5.5 on a nucleoside and a phosphate group donor. The phosphate group donor may be selected from polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof. The resulting nucleoside-5'-phosphate ester may subsequently be collected. As an alternative to using isolated acid phosphatase to catalyse the reaction, a microorganism transformed with a gene encoding a protein having acid phosphatase activity may be employed.

EP 0 857 788 A2

Printed by Jouve, 75001 PARIS (FR)

D ription

The pres nt invention relates to a method for producing nucleoside-5'-phosphate ester. Th pr s nt invention also relates to a novel acid phosphatase, a gene coding for th acid phosphatase, a recombinant DNA containing the gene, and a microorganism harboring the recombinant DNA which are useful to produce nucleoside-5'-phosphate ester. Nucleoside-5'-phosphate ester is useful as a seasoning, a pharmaceutical, and a raw material for producing such substances.

Methods for biochemically phosphorylating nucleoside to produce nucleoside-5'-phosphate ester by using the following phosphate group donors are known, including a method which uses p-nitrophenyphosphoric acid (Japanese Patent Publication No. 39-29858), a method which uses inorganic phosphoric acid (Japanese Patent Publication No. 42-1186), a method which uses polyphosphoric acid (Japanese Patent Laid-open No. 53-56390), a method which uses acetylphosphoric acid (Japanese Patent Laid-open No. 56-82098), and a method which uses adenosine triphosphate (ATP) (Japanese Patent Laid-open No. 63-230094). However, these methods have not been satisfactory to produce nucleoside-5'-phosphate ester efficiently and inexpensively because the substrates to be used are expensive, or because by-products are produced in the reaction.

Thus the present inventors have developed a method for efficiently producing nucleoside-5'-phosphate ester without by-producing 2'-, 3'-nucleotide isomers by allowing cells of a specified microorganism to act under an acidic condition on a nucleoside and a phosphate group donor selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof (Japanese Patent Laid-open No. 7-231793).

However, even this method has had the following drawbacks. Namely, for example, a part of the substrate is degraded during the reaction due to a nucleoside-degrading activity which unfortunately exists in a slight amount in the cells of the microorganism to be used. Moreover, if the reaction is continued, produced and accumulated nucleoside-5'-phosphate ester is degraded. Therefore, by-products are produced in a reaction solution, and it has been impossible to obtain a sufficient yield. In addition, the reaction cannot be performed if the substrate is added at a high concentration because of a low transphosphorylation activity per microbial cell.

In one embodiment, the present invention desirably provides a method for inexpensively and efficiently producing nucleoside-5'-phosphate ester. According to other embodiments of the present invention there are provided an enzyme, a gene coding for the enzyme, a recombinant DNA containing the gene, and a microorganism harboring the recombinant DNA which are useful for the method for producing nucleoside-5'-phosphate ester.

As a result of various investigations made by the present inventors in order to develop a method for producing nucleoside-5'-phosphate ester which is more efficient than the conventional methods, it has been found out that nucleoside-5'-phosphate ester maybe efficiently produced at a high yield by allowing an acid phosphatase purified from a cell-free extract of a microorganism to act under a condition of pH 3.0 to 5.5 on a nucleoside and a phosphate group donor selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof. Further, the present inventors have succeeded in obtaining wild type genes coding for acid phosphatases from various bacteria and genes coding for acid phosphatases having an increased affinity for the nucleoside as compared with the wild type acid phophatase by introducing a mutation into an acid phosphatase derived from a bacterium belonging to the genus Escherichia. Moreover, the present inventors have found out that productivity of nucleoside-5'-phosphate ester may be remarkably improved by expressing the gene in a large amount in accordance with genetic engineering techniques.

Further the present inventors have tried to prepare a mutant acid phosphatase with an increased temperature stability, with the intention that conducting a phosphate transfer reaction using the acid phosphatase at a higher temperature leads to a much more effective production of nucleoside-5'-phosphate because the reaction speed is elevated and the concentration of phosphate receiver in the reaction solution can be higher. Then the present inventors have succeeded in the preparation of a mutant acid phosphatase which has an increased temperature stability over mutant acid phosphatases described in Example 19 and can be employed in a reaction at a high temperature, and completed th present invention

Namely, the present invention provides a method for producing nucleoside-5'-phosphate ester comprising the steps of allowing an acid phosphatase having an increased affinity for a nucleoside and/or an increased temperature stability to act under a condition of pH 3.0 to 5.5 on a nucleoside and a phosphate group donor, preferably selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, acetylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof to produce nucleoside-5'-phosphate ester, and collecting it.

The t rm "acid phosphatas having an increased affinity for nucleosid " encompasses thos acid phosphatas s in which the affinity for nucleoside is higher in comparison with the wild type acid phosphatase. Preferred acid phosphatases have a Michaelis constant, Km, for transphosphorylation of the nucleoside, which is below 100mM.

The term "acid phosphatas having incr ase temperature stability" encompass s those acid phosphatas s which

retain more r sidual activity after treatment at el vated t mperature than does a corresponding wild type acid phosphatase. Preferably, the acid phosphatase retains more activity than the wild type after being held at 50°C for 30 minut s at pH 7.0. Particularly preferably, the acid phosphatase shows substantially no decrease in activity on treatment for 30 minutes at 50°C and pH 7.0.

In another aspect, the present invention provides a method for producing nucleoside-5'-phosphate ester comprising the steps of allowing a microorganism to act under a condition of pH 3.0 to 5.5 on a nucleoside and a phosphate group donor to produce nucleoside -5'-phosphate ester, and collecting it, wherein the microorgansm is transformed with a r combinant DNA comprising a gene encoding an acid phosphate having an increased affinity for the nucleoside and/or an increased temperature affinity.

In another aspect, the present invention provides mutant acid phosphatases having an increased affinity for a nucleoside and/or an increased temperature stability, genes coding for the acid phosphatases, recombinant DNAs containing the genes, and microorganisms harboring the recombinant DNA.

Embodiments of the invention are described below by way of example only and with reference to the accompanying figures, of which:

Fig. 1 illustrates a relationship between reaction pH and produced amount of 5'-inosinic acid in a reaction performed by using an enzyme derived from Morganella morganii.

Fig. 2 illustrates a relationship between reaction pH and produced amount of 5'-inosinic acid in a reaction performed by using an enzyme derived from Escherichia blattae.

Fig. 3 illustrates a restriction enzyme map of a chromosomal DNA fragment of Morganella morganii containing a gene coding for an acid phosphatase.

Fig. 4 illustrates produced amount of 5'-inosinic acid in a reaction performed by using a strain harboring phosphatase gene derived from Morganella morganii.

Fig. 5 illustrates a restriction enzyme map of a chromosomal DNA fragment of Escherichia blattae containing a gene coding for an acid phosphatase.

Fig. 6 illustrates a diagram showing produced amount of 5'-inosinic acid in a reaction performed by using a strain harboring the acid phosphatase gene derived from Escherichia blattae.

Fig. 7 illustrates produced amount of 5'-inosinic acid in reactions performed by using a strain harboring the wild type acid phosphatase gene and a strain harboring the mutant acid phosphatase gene derived from Escherichia blattae r spectively.

Fig. 8 illustrates produced amount of 5'-inosinic acid in a reaction performed by using a strain harboring the new mutant phosphatase gene derived from Escherichia blattae.

Fig. 9 illustrates a restriction enzyme map of a chromosomal DNA fragment derived from Enterobacter aerogenes which contains the gene coding for acid phosphatase.

Fig. 10 illustrates a restriction enzyme map of a chromosomal DNA fragment derived from Klebsiella planticola which contains the gene coding for acid phosphatase.

Fig. 11 illustrates a restriction enzyme map of a chromosomal DNA fragment derived from Serratia ficaria which contains the gene coding for acid phosphatase.

Fig.12 illustrates amino acid sequences in one-letter deduced from nucleotide sequences of acid phosphatases derived from Morganella morganii, Escherichia blattae, Providencia stuartii, Enterobacter aerogenes, Klebsiella planticola and Serratia ficaria. These amino acid sequences are illustrated in SEQ ID NOs: 4, 8, 22, 24, 26 and 28 in S quence Listing in three-letter. In the figuer, the amino acid residues which is common through the all amino acid sequences are marked with * below the sequence.

Fig. 13 illustrates the graph of the temperature stability of the acid phosphatase activity in the cell free extract solution prepared from a strain harboring the new mutant phosphatase gene derived from Escherichia blattae.

<1> Preparation of acid phosphatase

The acid phosphatase to be used in the present invention is not specifically limited provided that it catalyzes the reaction to produce nucleoside-5'-phosphate ester by phosphate group transfer to the nucleoside from the phosphate group donor, for example, selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, acethylphosphoric acid or a salt, and carbamyl phosphate or a salt thereof under the condition of pH 3.0 to 5.5. Such an acid phosphatase preferably includes those derived from microorganisms. In an especially preferred embodiment, the present invention uses an enzyme derived from a bacterium belonging to the genus Morganella, Escherichia, Providencia,

Enterobacter, Klebsiella or Serratia. Representative examples of such a bact rium include the following bacterial strains.

Mogan lla morganii NCIMB 10466

Morganella morganii IFO 3168
Morganella morganii IFO 3848
Escherichia blattae JCM 1650
Escherichia blattae ATCC 33429
Escherichia blattae ATCC 33430
Providencia stuartii ATCC 29851
Providencia stuartii ATCC 33672
Enterobacter aerogenes IFO 12010
Enterobacter aerogenes IFO 13534
Klebsiella planticola IFO 14939
Klebsiella planticola IAM 1133
Serratia ficaria IAM 13540
Serratia marcescens IAM 12143

It is noted that acid phosphatase (EC 3.1.3.2) is originally an enzyme which catalyzes a reaction to hydrolyze phosphate ester under an acidic condition, and it has a nucleotidase activity to degrade nucleoside-5'-phosphate ester produced by the transphosphorylation reaction (hereinafter, the nucleotidase activitiy is referred to as "phosphomonoesterase activity"). Even such an acid phosphatase can be used in the method for producing nucleoside-5'-phosphate ester of the present invention. However, in order to obtain nucleoside-5'-phosphate ester at a high yield, it is desirable to use the mutant acid phosphatase in which an affinity for a nucleoside in the transphosphorylation reaction onto the nucleoside is increased as compared with the wild type acid phosphatase produced by the bacteria as described above (hereinafter simply referred to as "mutant acid phosphatase", if necessary). Preferably the mutant acid phosphatase having a Km value below 100 is used.

The mutant acid phosphatase may be obtained by expressing a mutant gene obtained by directly mutating a gene coding for an acid phosphatase as described below. Alternatively, the mutant acid phosphatase may be also obtained by treating a microorganism which produces an acid phosphatase with irradiation of ultraviolet light or a mutating agent usually used for artificial mutation such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG), and cultivating a microorganism mutated to produce a mutant acid phosphatase having an increased affinity for a nucleoside and/or an increased temperature stability.

A protein having the acid phosphatase activity may be obtained from the microorganisms as described above by cultivating the microbial strain having the activity in an appropriate medium, harvesting proliferated microbial cells, disrupting the microbial cells to prepare a cell-free extract, and adequately purifying the protein therefrom.

The medium for cultivating the microorganism is not specifically limited, for which an ordinary medium may be available, containing an ordinary carbon source, a nitrogen source, inorganic ions, and optionally an organic nutrient source. The carbon source to be adequately used includes, for example, saccharides such as glucose and sucrose, alcohols such as glycerol, and organic acids. The nitrogen source to be used includes, for example, ammonia gas, aqueous ammonia, and ammonium salts. The inorganic ions to be adequately used if necessary include, for example, magnesium ion, phosphate ion, potassium ion, iron ion, and manganese ion. The organic nutrient source to be adequately used includes, for example, vitamins and amino acids as well as those containing them such as yeast extract, peptone, meat extract, corn steep liquor, casein hydrolysate, and soybean hydrolysate.

The cultivation condition is also not specifically limited. The microorganism may be cultivated, for example, under an aerobic condition for about 12 to 48 hours while appropriately controlling pH and temperature within ranges of pH 5 to 8 and temperature of 25 to 40 °C.

Proliferated microbial cells may be harvested from a culture liquid, for example, by centrifugation. The cell-free extract is prepared from the harvested microbial cells by using an ordinary method. Namely, the cell-free extract is obtained by disrupting the microbial cells by means of a method such as ultrasonic treatment, Dyno-mill, and French Press, and removing cell debris by centrifugation.

The acid phosphatase is purified from the cell-free extract by using an adequate combination of techniques usually used for enzyme purification such as ammonium sulfate fractionation, ion exchange chromatography, hydrophobic chromatography, affinity chromatography, gel filtration chromatography, and isoelectric purification. As for the precipitation, it is not necessarily indispensable to completely purify the acid phosphatase. It is sufficient to achieve removal of contaminants such as an enzyme which participates in degradation of nucleoside as the substrate.

<2> Preparation of acid phosphatase gene

A DNA fragment, which contains a structural gene coding for the protein having the acid phosphatase activity, can be cloned starting from, for example, cells of the microorganism having the enzyme activity. The cloning method includes, for exampl , a method in which a chromosomal gene expr ssion library is screened by using the enzyme

activity as an index, a method in which an antibody against the protein is prepared to screen a chromosomal gene expression library, and a method in which an amino acid sequence such as an N-terminal sequence of the purified protein is analyzed, on the basis of which a probe is prepared to screen a gene library.

Specifically, the gene coding for the acid phosphatase of Morganella morganii, Escherichia blattae, Providencia stuartii, Enterobacter aerogenes, Klebsiella planticola, Serratia ficaria or Serratia marcescens described above can be cloned by preparing a chromosomal gene expression library of each of the microorganisms, and screening the library by using the phosphatase activity as an index.

Namely, a chromosomal gene expression library can be prepared by firstly preparing chromosomal DNA from Morganella morganii or Escherichia blattae, partially degrading it with an appropriate restriction enzyme, subsequently ligating it with a vector autonomously replicable in Escherichia coli, and transforming Escherichia coli with the obtained recombinant DNA. A wide variety of restriction enzymes can be used for digesting chromosomal DNA by adjusting the digestion reaction time to adjust the degree of digestion. Any vector may be used for cloning the gene provided that it is autonomously replicable in Escherichia coli. It is possible to use, for example, pUC19, pUC118, pHSG298, pBR322, and pBluescript II.

The vector may be ligated with the DNA fragment containing the gene coding for the acid phosphatase to prepare the recombinant DNA by previously digesting the vector with the same restriction enzyme as that used for digesting chromosomal DNA, or with a restriction enzyme which generates a cleaved edge complementary with a cleaved edge of the chromosomal DNA fragment, and ligating it with the DNA fragment by using ligase such as T4 DNA ligase. Any microbial strain may be used as a recipient for the prepared recombinant DNA provided that it is appropriate for replication of the vector. It is possible to use, for example, microbial strains of Escherichia coli such as HB101, JM109, and DH5.

Transformants thus obtained are grown on an agar medium to form their colonies. After that, when a reaction solution containing p-nitrophenylphosphoric acid is poured onto a surface of the medium to perform a reaction, then a strain, which has expressed the phosphatase activity, liberates p-nitrophenol and exhibits a yellow color. A transformant, which harbors a DNA fragment containing the gene coding for the objective acid phosphatase, can be selected by performing the reaction described above under an acidic condition, and selecting the transformant by using the color development as an index.

After that, a recombinant DNA is recovered from the selected transformant to analyze the structure of the DNA fragment containing the gene coding for the acid phosphatase ligated with the vector. A nucleotide sequence of the gene coding for the acid phosphatase is shown in SEQ ID NO: 2 in Sequence Listing in the case of a gene derived from Morganella morganii NCIMB 10466, SEQ ID NO: 6 in Sequence Listing in the case of a gene derived from Escherichia blattae JCM 1650, SEQ ID NO: 21 in Sequence Listing in the case of a gene derived from Providencia stuartii ATCC 29851, SEQ ID NO: 23 in Sequence Listing in the case of a gene derived from Enterobacter aerogenes IFO 12010, SEQ ID NO: 25 in Sequence Listing in the case of a gene derived from Klebsiella planticola IFO14939, or SEQ ID NO: 27 in Sequence Listing in the case of a gene derived from Serratia ficaria IAM 13540.

The deduced amino acid sequences of the acid phosphatases encoded by the above genes are illustrated in SEQ ID NO: 4, 8, 22, 24, 26 and 28.

Variants of the acid phosphatases encoded by the above genes and having increased affinity for nucleoside and/or increased temperature stability, are preferably used for the present invention. Acid phosphatase comprising an amino acid sequence which is substantially identical with an amino acid sequence of any one of the acid phosphatases encoded by the above genes is also preferably used for the present invention. The term "substantially identical" means that amino acid sequences of the acid,phosphatases may have substitution, deletion, insertion or transition of one or a plurality of amino acid residues without losing an activity to produce nucleoside-5'-phosphate ester (hereinafter referred to as "transphosphorylation activity"). Preferably, the variants have a Michaelis constant Km below 100°C as discussed above and experience substantially no loss of activity when held at 50°C and pH7 for 30 minutes.

<3> Preparation of gene coding for mutant acid phosphatase

The wild type acid phosphatase obtained as described above has a phosphomonoesterase activity. Therefore, the phosphomonoesterase activity may serve as a factor to cause accompanying degradation of the product as the reaction time passes in the production of nucleoside-5'-phosphate ester, resulting in decrease in reaction yield. In order to overcome such a circumstance, it is advantageous to cause artificial mutation on the gene coding for the acid phosphatase so that an affinity for a nucleoside is increased.

Further conducting a phosphate transfer reaction by the acid phosphatase at a higher temperature leads to a much more effective production of nucleoside-5'-phosphate because the reaction speed is elevated and the concentrattion of a phosphate reciever in the reaction solution can be higher. For the purpose it is advantageous to cause artificial mutation on the gene coding for the acid phosphatase so that a temperatre stability is increased.

Methods for site-directed mutagenesis for causing objective mutation at an objective site of DNA include, for ex-

ample, a method using PCR (Higuchi, R., 61, in PCR t chnology, Erlich, H. A. Eds., Stockton pr ss (1989); carter, P., Meth. in Enzymol., 154, 382 (1987)), and a method using phage (Kramer, W. and Frits, H. J., Meth. in Enzymol., 154, 350 (1987); Kunkel, T. A. et al., Meth. in Enzymol., 154, 367 (1987)).

The mutant acid phosphatase having the increas d affinity for the nucleoside is exemplified by the acid phosphatase comprising an amino acid sequence which is substantially identical with an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 22, 24, 26 and 28 in Sequence Listing, and has mutation which increases the affinity for the nucleoside compared to the wild type acid phosphatase. Concretely, the mutant acid phosphatase is exemplified, for the enzyme derived from Escherichia blattae JCM 1650, by one in which the 74th glycine residue and/or the 153th isoleucine residue is substituted with another amino acid residue in an amino acid sequence illustrated in SEQ ID NO: 8 in Sequence Listing. In Examples described below, a gene coding for mutant acid phosphatase is illustrated as an example in which the 74th glycine residue is substituted with an aspartic acid residue, and the 153th isoleucine residue is substituted with a threonine residue.

Further mutations selected from the group consisting of substitutions of the 63rd leucine residue, the 65th alanine residue, the 66th glutamic acid residue, the 69th aspartic acid residue, the 71st serine residue, the 72nd serine residue, the 85th serine residue, the 92nd alanine residue, the 94th alanine residue, the 116th aspartic acid residue, the 130th serine residue, the 135th threonine residue and/or the 136th glutamic acid residue with another amino acid in SEQ ID NO: 8 in Sequence Listing further increase the affinity for the nucleoside of the acid phosphatase.

The mutant acid phosphatase having the increased temperature stability is exemplified by the acid phosphatase comprising an amino acid sequence which is substantially identical with an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 22, 24, 26 and 28 in Sequence Listing, and has mutation which increases the temperature stability of wild type acid phosphatase. Concretely, the mutant acid phosphatase is exemplified, for the enzyme derived from Escherichia blattae JCM 1650, by one in which the 104th glutamic acid residue and/or the 151st threonine residue is substituted with another amino acid residue in an amino acid sequence illustrated in SEQ ID NO: 8 in Sequence Listing. In Examples described below, a gene coding for mutant acid phosphatase is illustrated as an example in which the 104th glutamic acid residue is substituted with a glycine residue, and the 151st threonine residue is substituted with an alanine residue.

Therefore, the nucleotide may be substituted at the specified site of the wild type gene in accordance with the site-directed mutagenesis method described above so that these mutant acid phosphatases are encoded. The mutation to increase the affinity for the nucleoside is desirably a type of mutation by which the activity to produce nucleoside-5'-phosphate ester is not substantially lowered in comparison with wild type acid phosphatase. However, even in the case that the activity to produce nucleoside-5'-phosphate ester is lowered, it will be sufficient if degree of decrease of phosphomonoesterase activity is larger than that of the activity to produce nucleoside-5'-phosphate ester, with the result that a ratio of phosphomonoesterase activity to the activity to produce nucleoside-5'-phosphate ester of the mutant acid phosphatase is lowered in comparison with the wild type acid phosphatase. As for the degree of increase in the affinity for the nucleoside, the Km value to the nucleoside in the transphosphorylation reaction is preferably below 100.

The mutation which increases the temperature stability means one which has more residual activity after a temperature treatment than the wild type acid phosphatase has. The degree of the temperature stability increase is preferably the one that does not cause the decrease in an activity with the treatment at pH 7.0, 50°C, 30 minutes.

As illustrated below in the embodiments, the amino acid sequence of the acid phosphatase of Escherichia blattae JCM 1650 is highly homologous to that of Morganella morganii NCIMB 10466, and the 72nd glycine residue, the 102th glutamic acid residue, the 149th threonine residue and the 151th isoleucine residue in an amino acid sequence illustrated in SEQ ID NO: 4 correspond to the 74th glycine residue, the 104th glutamic acid residue, the 151st threonine residue and the 153rd isoleucine residue in an amino acid sequence illustrated in SEQ ID NO: 8 respectively. Further, in addition to Escherichia blattae JCM 1650, amino acid sequences of acid phosphatases derived from microorganisms such as Providencia stuartii ATCC 29851, Enterobacter aerogenes IFO 12010, Klebsiella planticola IFO 14939, and Serratia ficaria IAM 13450 have high homology with that of Morganella morganii NCIMB 10466, and amino acid sequences of these acid phosphatases include amino acids resudues each of which corresponds to the 72nd glycine r sidue, the 102nd glutamic acid residue, the 149th threonine residue and the 151st isoleucine residue in an amino acid sequence illustrated in SEQ ID NO: 4 respectively. Therefore, genes coding for mutant acid phosphatases derived from these microorganisms may be obtained as described above. The 92nd glycine residue, the 122nd glutamic acid residue, the 169th threonine residue and the 171st isoleucine residue in the amino acid sequence of the acid phosphatase derived from Providencia stuartii ATCC 29851, Enterobacter aerogenes IFO 12010 or Klebsiella planticola IFO 14939 illustrated in SEQ ID NO: 22, 24 or 26, and the 88th glycine residue, the 118th glutamic acid residue, the 165th threonine residu and the 167th isoleucine residue in the amino acid sequenc of the acid phosphatase derived from Serratia ficaria IAM 13450 illustrated in SEQ ID NO: 28 respectively correspond to the 72nd glycine residue, the 102nd glutamic acid residue, the 149th threonine residue and the 151st isoleucine residue in an amino acid sequence illustrated in SEQ ID NO: 4.

The result of comparing the amino acid sequences of the above acid phophatase is illustrated in Fig. 12. Based on Fig. 12, it is decided which amino acid residue of one acid phosphatase corresponds to another amino acid residue of another acid phosphatase.

<4> Introduction of acid phosphatase gene into host

A recombinant microorganism for expressing the acid phosphatase activity at a high level can be obtained by introducing the DNA fragment containing the gene coding for the protein having the acid phosphatase activity obtained as described above into cells of a host after recombining the DNA fragment again with an appropriate vector. In such a procedure, the wild type acid phosphatase is expressed by using the gene coding for the wild type acid phosphatase, while the mutant acid phosphatase is expressed by using the gene coding for the mutant acid phosphatase.

The host includes the microbial strains of Escherichia coli such as HB101, JM109, and DH5 described above. Other than these strains, all bacteria can be utilized as the host provided that a replication origin of constructed recombinant DNA and the acid phosphatase gene make their functions, the recombinant DNA is replicable, and the acid phosphatase gene is expressible. One of the most preferred hosts is Escherichia coli JM109.

The vector for incorporating the gene coding for the acid phosphatase thereinto is not specifically limited provided that it is replicable in the host. When Escherichia coli is used as the host, the vector may be exemplified by plasmids autonomously replicable in this bacterium. For example, it is possible to use ColE1 type plasmids, p15A type plasmids, R factor type plasmids, and phage type plasmids. Such plasmids specifically include, for example, pBR322 (Gene, 2, 95 (1977)), pUC19 (Gene, 33, 103 (1985)), pUC119 (Methods in Enzymology, 153, 3 (1987)), pACYC184 (J. Bacteriol., 134, 1141 (1978)), and pSC101 (Proc. Natl. Acad. Sci. U.S.A., 70, 3240 (1973)).

When the DNA fragment containing the gene coding for the acid phosphatase contains a promoter which is functional in the host, the DNA fragment may be ligated with the vector as it is. When the DNA fragment does not contain such a promoter, another promoter which works in the host microorganism such as lac, trp, and PL may be ligated at a position upstream from the gene. Even when the DNA fragment contains the promoter, the promoter may be substituted with another promoter in order to efficiently express the gene coding for the acid phosphatase.

There is no special limitation for a method for introducing, into the host, the recombinant DNA constructed by ligating the vector with the DNA fragment containing the gene coding for the acid phosphatase. The recombinant DNA may be introduced into the host by using an ordinary method. When Escherichia coli is used as the host, it is possible to use, for example, a calcium chloride method (J. Mol. Biol., 53, 159 (1970)), a method of Hanahan (J. Mol. Biol., 166, 557 (1983)), an SEM method (Gene, 96, 23 (1990)), a method of Chung et al. (Proc. Natl. Acad. Sci. U.S.A., 86, 2172 (1989)), and electroporation (Nucleic Acids Res., 16, 6127 (1988)).

The acid phosphatase gene may be inserted into the autonomously replicable vector DNA, which may be introduced into the host so that it is harbored by the host as extrachromosomal DNA as described above. Alternatively, the acid phosphatase gene may be incorporated into chromosome of the host microorganism in accordance with a method which uses transduction, transposon (Biotechnol., 1, 417 (1983)), Mu phage (Japanese Patent Laid-open No. 2-109985), or homologous recombination (Experiments in Molecular Genetics, Cold Spring Harbor Lab. (1972)).

<5> Expression of acid phosphatase gene by recombinant microorganism

The transformant obtained as described above, into which the recombinant DNA containing the gene coding for the acid phosphatase has been introduced, is capable of expressing the acid phosphatase activity at a high level in its cells by cultivating it in an appropriate medium containing a carbon source, a nitrogen source, inorganic ions, and optionally an organic nutrient source. The carbon source to be adequately used includes, for example, carbohydrates such as glucose, alcohols such as glycerol, and organic acids. The nitrogen source to be used includes, for example, ammonia gas, aqueous ammonia, and ammonium salts. The inorganic ions to be adequately used if necessary include, for example, magnesium ion, phosphate ion, potassium ion, iron ion, and manganese ion. The organic nutrient source to be adequately used includes, for example, vitamins and amino acids as well as those containing them such as yeast extract, peptone, meat extract, corn steep liquor, casein hydrolysate, and soybean hydrolysate. The amount of expression of the acid phosphatase activity may be increased by adding, to the medium, an expression-inducing agent depending on a promoter such as IPTG (isopropyl-β-D-thiogalactopyranoside).

The cultivation condition is also not specifically limited. The cultivation may be performed, for example, under an aerobic condition for about 12 to 48 hours while appropriately controlling pH and temperature within ranges of pH 5 to 8 and temperature of 25 to 40 °C.

After that, microbial cells are harvested from a culture, and a cell-free extract is obtained by disruption, from which the acid phosphatase can be purified. The purification is performed by using an appropriate combination of techniques usually used for enzyme purification such as those described in the aforementioned item <1>. As for the purification, it is not necessarily indispensable to completely purify the acid phosphatase. It is sufficient to achieve removal of

contaminants such as an enzyme which participates in degradation of nucleosid as the substrate.

<6> Production of nucl oside-5'-phosphate ster

Nucleoside-5'-phosphate ester can be produced in a reaction mixture by allowing the acid phosphatase obtained as described above, and having increased affinity for nucleoside and/or increased temperature stability, such as the mutant acid phosphatase obtained by expressing the gene in a large amount in accordance with the genetic engineering technique as described in the item <5> to make contact and cause the reaction of a nucleoside with a phosphate group donor selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, acetylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof. In order to achieve a high productivity in this reaction, it is important to adjust pH of the reaction solution to be weakly acidic in a range of 3.0 to 5.5.

When the gene coding for the acid phosphatase is expressed in a large amount by means of the genetic engineering technique, especially when the gene coding for the mutant acid phosphatase having the increased affinity for the nucleoside is expressed in a large amount, then it is also possible to produce nucleoside-5'-phosphate ester inexpensively and efficiently by using a culture containing microbial cells of the transformant, the microbial cells separated and harvested from the culture, or a product obtained from the microbial cells in accordance with, for example, an immobilizing treatment, an acetone treatment, or a lyophilizing treatment, instead of the purified acid phosphatase.

The nucleoside to be used includes, for example, purine nucleosides such as inosine, guanosine, adenosine, xanthosine, purine riboside, 6-methoxypurine riboside, 2,6-diaminopurine riboside, 6-fluoropurine riboside, 6-thiopurine riboside, 2-amino-6-thiopurine riboside, and mercaptoguanosine; and pyrimidine nucleosides such as uridine, cytidine, 5-aminouridine, 5-hydroxyuridine, 5-bromouridine, and 6-azauridine. As a result of the reaction, these natural type nucleosides and nonnatural type nucleosides are specifically phosphorylated at their 5'-positions, and corresponding nucleoside-5'-phosphate esters are produced respectively.

The nucleoside is desirably added to the reaction solution at a concentration of 1 to 20 g/dl. In the case of use of a nucleoside which is scarcely soluble in water, the reaction yield may be improved by adding boric acid or a surfactant such as dimethyl sulfoxide.

When the nucleoside is produced by fermentation, the fermentation medium after the fermentation as such can be added to the phosphorylation reaction liquid. When an element decomposing the nucleoside-5'-phosphate ester is included in the medium, a purification step is preferably employed so that said element is removed.

As for the phosphate group donor to be used, those usable as the polyphosphoric acid or the salt thereof include, for example, pyrophosphoric acid, tripolyphosphoric acid, trimetaphosphoric acid, tetrametaphosphoric acid, hexametaphosphoric acid, mixtures thereof, sodium salts thereof, potassium salts thereof, and mixtures of these salts. Those usable as the phenylphosphoric acid or the salt thereof include, for example, disodium phenylphosphate, dipotassium phenylphosphate, O,O-diphenylphosphoric acid anhydride, and mixtures thereof. Those usable as the carbamyl phosphate or the salt thereof include, for example, disodium carbamyl phosphate, dipotassium carbamyl phosphate, diammonium carbamyl phosphate, dilithium carbamyl phosphate, and mixtures thereof. Those usable as the acetylphosphoric acid or the salt thereof include, for example, lithium potassium acetylphosphate. The concentration at which the phosphate group donor is used is determined by the concentration of the nucleoside as the phosphate group acceptor. The phosphate group donor is usually used in an amount which is 1 to 5 times that of the nucleoside.

A preferred result is obtained in the reaction usually at a temperature of 20 to 60 °C, preferably 30 to 40 °C at a pH on a weakly acidic side of 3.5 to 6.5, preferably 4.0 to 5.0. A reaction temperature when using the mutant acid phosphatase with an increased temperature stability is 20 to 70 °C, preferably 30 to 60 °C. The reaction may be performed by adopting any one of a stationary method and an agitating method. The reaction time defers depending on the condition such as the activity of the enzyme to be used and the substrate concentration, however, it is 1 to 100 hours.

The nucleoside-5'-phosphate ester thus produced may be collected and separated from a mixture after completion of the reaction by adopting a method to use a synthetic resin for adsorption, a method to use a precipitating agent, and other ordinary methods for collection and separation.

[EXAMPLE]

Embodiments of the present invention will be specifically explained below with reference to Examples, however, the present invention is not limited to these Examples.

The transphosphorylation activity was measured under the following condition using inosine as a substrate. The r action was performed at pH 5.0 at 30 °C for 10 minutes in a reaction solution (1 ml) containing 40 µmol/ml of inosine, 100 µmol/ml of sodium pyrophosphate, 100 µmol/ml of sodium acetate buffer (pH 5.0), and an enzyme. The reaction was stopped by adding 200 µl of 2 N hydrochloric acid. After that, precipitates were r moved by centrifugation. Then, 5'-In sinic acid produced by the transphosphorylation reaction was quantitatively measured. An amount of enzyme to

produc 1 µmol of 5'-inosinic acid per 1 minute under this standard reaction condition was defined as 1 unit.

The phosphomonoesterase activity was measured under the following condition using 5'-inosinic acid as a substrate. The reaction was perform d at 30 °C for 10 minutes in a reaction solution (1 ml) containing 10 µmol/ml of 5'-inosinic acid, 100 µmol/ml of MES/NaOH buffer (pH 6.0), and an enzyme. The reaction was stopped by adding 200 µl of 2 N hydrochloric acid. After that, precipitates were removed by centrifugation. Then, inosine produced by the hydrolytic reaction was quantitatively measured. An amount of enzyme to produce 1 µmol of inosine per 1 minute under this standard reaction condition was defined as 1 unit.

Inosine and 5'-inosinic acid were analyzed under the following condition by means of high-performance liquid chromatography (HPLC).

Column:	Cosmosil 5C18-AR (4.6 x 150 mm) [produced by nacalai tesque];
Mobile phase:	5 mM potassium phosphate buffer (pH 2.8)/methanol = 95/5;
Flow rate:	1.0 ml/min;
Temperature:	room temperature;
D tection:	UV 245 nm.

Incidentally, in the reaction to produce nucleoside-5'-phosphate esters using nucleosides other than inosine as raw materials, the nucleosides as raw materials and produced nucleoside-5'-phosphate esters were analyzed by HPLC as described above.

Example 1: Purification and Characterization of Acid Phosphatase Derived from Morganella morganii

A nutrient medium (pH 7.0, 50 ml) containing 1 g/dl of peptone, 0.5 g/dl of yeast extract, and 1 g/dl of sodium chloride was poured into Sakaguchi flasks (500 ml), which was sterilized at 120 °C for 20 minutes. A slant culture of Morganella morganii NCIMB 10466 was inoculated to each of the flasks once with a platinum loop, which was cultivated at 30 °C for 16 hours with shaking. Microbial cells (about 3,000 g), which were harvested from a culture by centrifugation, were suspended in 100 mM potassium phosphate buffer (1 L, pH 7.0). A ultrasonic treatment was performed at 4 °C for 20 minutes to disrupt the microbial cells. The treated suspension was centrifuged to remove its insoluble fraction. Thus a cell-free extract was prepared.

Ammonium sulfate was added to the cell-free extract so that 30 % saturation was achieved. Appeared precipitate was removed by centrifugation, and then ammonium sulfate was further added to supernatant so that 60 % saturation was achieved. Appeared precipitate was collected by centrifugation, and it was dissolved in 100 mM potassium phosphate buffer.

This crude enzyme solution was dialyzed four times against 5 L of 100 mM potassium phosphate buffer (pH 7.0), and then it was applied to a DEAE-Toyopeal 650M column (ϕ 4.1 x 22 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7.0), followed by washing with 800 ml of 20 mM potassium phosphate buffer (pH 7.0). The transphosphorylation activity was found in a fraction which passed through the column, and thus the fraction was recovered.

The fraction was added with ammonium sulfate so that 35 % saturation was achieved, which was adsorbed to a Butyl-Toyopeal column (ϕ 3.1 x 26 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7.0) containing ammonium sulfate at 35 % saturation. Elution was performed by using a linear concentration gradient from 35 % saturation to 20 % saturation in potassium phosphate buffer (pH 7.0).

Active fractions were collected and dialyzed against 1 L of 50 mM potassium phosphate buffer (pH 7.0), followed by being applied to a hydroxyapatite column (ϕ 5 x 6.5 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.0). Elution was performed by using a linear concentration gradient from 50 mM to 300 mM of potassium phosphate buffer (pH 7.0).

Active fractions were collected and concentrated by ultrafiltration. This enzyme solution was applied into a HiLoad TM 16/60 Superdex 200 column (produced by Pharmacia). Elution was performed at a flow rate of 1.0 ml/minute by using 50 mM potassium phosphate buffer containing 100 mM sodium chloride.

In accordance with the procedure as described above, the enzyme exhibiting the transphosphorylation activity was purified from the cell-free extract consequently about 550-fold at a recovery ratio of about 10 %. The specific activity and the recovery ratio in this purification process are shown in Table 1. This enzyme sample was homogeneous on SDS-polyacrylamide gel electrophoresis.

Table 1

Step Recovery	Total activity (unit)	Total protein (mg)	Specific activity (unit/mg)	ratio (%)
1. Cell-fre extract	597	127,200	0.005	100

Table 1 (continued)

Step Recovery	Total activity (unit)	Total protein (mg)	Specific activity (unit/mg)	ratio (%)
2. Ammonium sulfate fractionation (30 to 60 %)	568	122,210	0.005	95
3. DEAE-Toyopearl	517	36,498	0.014	87
4. Butyl-Toyopearl	394	1,121	0.351	66
5. Hydroxyapatite	112	50	2.244	19
6. Superdex 200	63	24	2.630	10

The purified enzyme had the following properties.

(1) Action: Phosphate group is transferred from a phosphate group donor such as polyphosphoric acid to nucleoside, and nucleoside-5'-phosphate ester is produced. Reversely, this enzyme also exhibits an activity to hydrolyze phosphate ester.
(2) Substrate specificity: Those which serve as the phosphate group donor in the transphosphorylation reaction include, for example, pyrophosphoric acid, tripolyphosphoric acid, trimetaphosphoric acid, tetrametaphosphoric acid, hexametaphosphoric acid, disodium phenylphosphate, dipotassium phenylphosphate, O,O-diphenylphosphoric acid anhydride, disodium carbamyl phosphate, dipotassium carbamyl phosphate, diammonium carbamyl phosphate, and dilithium carbamyl phosphate. Those which serve as the phosphate group acceptor include, for example, purine riboside, inosine, guanosine, adenosine, xanthosine, uridine, and cytidine. On the other hand, those which undergo the action in the phosphate ester hydrolytic reaction include, for example, inorganic phosphoric acid such as pyrophosphoric acid, tripolyphosphoric acid, trimetaphosphoric acid, tetrametaphosphoric acid, hexametaphosphoric acid; phosphate ester such as disodium phenylphosphate, dipotassium phenylphosphate, O,O-diphenylphosphoric acid anhydride, disodium carbamyl phosphate, dipotassium carbamyl phosphate, diammonium carbamyl phosphate, and dilithium carbamyl phosphate; and 5'-nucleotide such as 5'-purine ribotide, 5'-inosinic acid, 5'-guanylic acid, 5'-adenylic acid, 5'-xanthylic acid, 5'-uridylic acid, and 5'-cytidylic acid.
(3) Optimum pH: 5.2 (transphosphorylation reaction), 6.5 (phosphate ester hydrolytic reaction).
(4) pH stability: pH 3.0 to 12.0 (treatment at 30 °C for 60 minutes).
(5) Optimum temperature: about 35 °C.
(6) Temperature stability: stable up to 30 °C (treatment at pH 7.0 for 30 minutes).
(7) Effect of the addition of metal ion and inhibitor: This enzyme exhibits no activation phenomenon relevant to its activity by addition of any metal ion. The activity is inhibited by Ag^{2+}, Pb^{2+}, Hg^{2+}, and Cu^{2+}. The activity is also inhibited by iodoacetic acid.
(8) Molecular weight: A calculated molecular weight is about 190,000 in accordance with high-performance liquid chromatography (TSKgel G-3000SW, produced by Toyo Soda).
(9) Subunit molecular weight: A calculated subunit molecular weight is about 25,000 in accordance with SDS-polyacrylamide gel electrophoresis.

This enzyme exhibits not only the activity to transfer phosphate group to nucleoside, but also the activity to reversely hydrolyze phosphate ester. Moreover, this enzyme exhibits the phosphate ester hydrolytic activity (phosphomonoestrase activity) which is higher than the transphosphorylation activity by not less than 20 times. Other properties are well coincident with those of a known acid phosphatase produced by a bacterium belonging to the genus Morganella (Microbiology, 140, 1341-1350 (1994)). Accordingly, it has been clarified that this enzyme is an acid phosphatase.

Sodium pyrophosphate (10 g/dl) and inosine (2 g/dl) were dissolved in sodium acetate buffers each having pH of 5.5, 5.0, 4.5, 4.0, and 3.5, to which the enzyme sample described above was added so that a concentration of 50 units/dl was obtained. The reaction mixture was incubated at 30 °C for 6 hours while maintaining each pH, and the amount of produced 5'-inosinic acid was measured along with passage of time. Produced inosinic acid contained only 5'-inosinic acid. By-production of 2'-inosinic acid and 3'-inosinic acid was not observed at all. A result is shown in Fig. 1. The v locity of 5'-inosinic acid production was maximum at pH 5.0. However, the maximum accumulated amount of 5'-inosinic acid was higher at lower pH. The reaction condition at pH 4.0 was most efficient for production of 5'-inosinic acid, in which 5'-inosinic acid was produced and accumulated in an amount of 2.60 g/dl by performing the reaction for 3 hours.

Example 2: Phosphorylation Reaction of Various Nucl osides by Acid Phosphatase Sample Derived from Morganella morganii

Sodium pyrophosphate (10 g/dl) and inosine, guanosine, uridine, or cytidine (2 g/dl) as a phosphate group acceptor were dissolved in sodium acetate buffer (pH 4.0), to which the enzyme sample prepared in Example 1 was added so that its concentration was 50 units/dl. The reaction mixture was incubated at 30 °C for 3 hours while maintaining pH at 4.0. The amount of nucleoside-5'-ester produced by the reaction is shown in Table 2.

Produced nucleotide contained only nucleoside-5'-ester. By-production of nucleoside-2'-ester and nucleoside-3'-ester was not observed at all.

Table 2

Nucleoside	Product	Produced amount (g/dl)
Inosine	5'-inosinic acid	2.60
Guanosine	5'-guanylic acid	1.90
Uridine	5'-uridylic acid	1.30
Cytidine	5'-cytidylic acid	0.98

Example 3: Production of 5'-Inosinic acid from Various Phosphate Compounds as Phosphate Group Donors by Acid Phosphatase Sample Derived from Morganella morganii

Inosine (2 g/dl) and sodium tripolyphosphate, sodium polyphosphate (trade name: Polygon P, produced by Chiyoda Chemical), disodium phenylphosphate, or disodium carbamyl phosphate (10 g/dl) as a phosphate group donor were dissolved in sodium acetate buffer (pH 4.0), to which the enzyme sample prepared in Example 1 was added so that its concentration was 50 units/dl. The reaction mixture was incubated at 30 °C for 3 hours while maintaining pH at 4.0. The amount of 5'-inosinic acid produced by the reaction is shown in Table 3.

5'-Inosinic acid was efficiently produced and accumulated by using any of the phosphate group donors. However, the accumulated amount of 5'-inosinic acid was the highest when sodium polyphosphate was used as the phosphate group donor.

Table 3

Phosphate group donor	Produced 5'-inosinic acid (g/dl)
Sodium tripolyphosphate	2.10
Sodium polyphosphate	2.72
Disodium phenylphosphate	2.33
Disodium carbamyl phosphate	2.54

Example 4: Purification and Characterization of Acid Phosphatase Derived from Escherichia blattae

A nutrient medium (pH 7.0, 50 ml) containing 1 g/dl of peptone, 0.5 g/dl of yeast extract, and 1 g/dl of sodium chloride was poured into Sakaguchi flasks (500 ml), which was sterilized at 120 °C for 20 minutes. A slant culture of Escherichia blattae JCM 1650 was inoculated to each of the flasks once with a platinum loop, which was cultivated at 30 °C for 16 hours with shaking. Microbial cells were harvested from a culture by centrifugation. The microbial cells (about 3,300 g) were suspended in 100 mM potassium phosphate buffer (1 L, pH 7.0). A ultrasonic treatment was performed at 4 °C for 20 minutes to disrupt the microbial cells. The treated suspension was centrifuged to remove its insoluble fraction. Thus a cell-free extract was prepared.

Ammonium sulfate was added to the cell-free extract so that 30 % saturation was achieved. Appeared precipitate was removed by centrifugation, and then ammonium sulfate was further added to supernatant so that 60 % saturation was achieved. Appeared precipitate was recovered by centrifugation, and it was dissolved in 100 mM potassium phosphate buffer.

This crude enzyme solution was dialyzed four times against 5 L of 100 mM potassium phosphate buffer (pH 7.0), and then it was applied to a DEAE-Toyopeal 650M column (ϕ 6.2 x 35 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7.0), followed by washing with 20 mM potassium phosphate buffer (pH 7.0). The transphosphorylation activity was found in a fraction which passed through the column, and thus the fraction was collected.

Th active fraction was add d with ammonium sulfat so that 35 % saturation was achieved, which was applied to a Butyl-Toyopeal column (ϕ 5.0 x 22.5 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7.0) containing

ammonium sulfate at 35 % saturation. Elution was performed by using a linear concentration gradient from 35 % saturation to 20 % saturation in potassium phosphate buffer (pH 7.0).

Active fractions were collected and dialyzed against 1 L of 100 mM potassium phosphate buffer (pH 7.0), followed by being applied to a hydroxyapatite column (ϕ 3.0 x 7.0 cm) equilibrated with 100 mM potassium phosphate buffer (pH 7.0). Elution was performed by using a linear concentration gradient from 50 mM to 100 mM of potassium phosphate buffer (pH 7.0), and active fractions were collected.

This enzyme solution was dialyzed against 1 L of 10 mM potassium phosphate buffer (pH 6.0), followed by being applied to a CM-Toyopearl column (ϕ 2.0 x 14.0 cm) equilibrated with 10 mM potassium phosphate buffer (pH 6.0). Elution was performed by using a linear concentration gradient in potassium phosphate buffer (pH 6.0) containing from 0 mM to 300 mM potassium chloride. Active fractions eluted from the column were collected.

In accordance with the procedure as described above, the enzyme exhibiting the transphosphorylation activity was purified from the cell-free extract consequently about 600-fold at a recovery ratio of about 16 %. The specific activity and the recovery ratio in this purification process are shown in Table 4. This enzyme sample was homogeneous on SDS-polyacrylamide gel electrophoresis.

Table 4

Step	Total activity (unit)	Total protein (mg)	Specific activity (unit/mg)	Recovery ratio (%)
1. Cell-free extract	365	160,650	0.002	100
2. Ammonium sulfate fractionation (30 to 60 %)	340	138,895	0.002	93
3. DEAE-Toyopearl	318	30,440	0.010	87
4. Butyl-Toyopearl	232	661	0.347	63
5. Hydroxyapatite	96	96	1.000	26
6. CM-Toyopearl	59	43	1.365	16

The purified enzyme had the following properties.

(1) Action: Phosphate group is transferred from a phosphate group donor such as polyphosphoric acid to nucleoside, and nucleoside-5'-phosphate ester is produced. Reversely, this enzyme also exhibits an activity to hydrolyze phosphate ester.

(2) Substrate specificity: Those which serve as the phosphate group donor in the transphosphorylation reaction include, for example, pyrophosphoric acid, tripolyphosphoric acid, trimetaphosphoric acid, tetrametaphosphoric acid, hexametaphosphoric acid, disodium phenylphosphate, dipotassium phenylphosphate, O,O-diphenylphosphoric acid anhydride, disodium carbamyl phosphate, dipotassium carbamyl phosphate, diammonium carbamyl phosphate, and dilithium carbamyl phosphate. Those which serve as the phosphate group acceptor include, for example, purine riboside, inosine, guanosine, adenosine, xanthosine, uridine, and cytidine. On the other hand, those which undergo the action in the phosphate ester hydrolytic reaction include, for example, inorganic phosphoric acid such as pyrophosphoric acid, tripolyphosphoric acid, trimetaphosphoric acid, tetrametaphosphoric acid, hexametaphosphoric acid; phosphate ester such as disodium phenylphosphate, dipotassium phenylphosphate, O,O-diphenylphosphoric acid anhydride, disodium carbamyl phosphate, dipotassium carbamyl phosphate, diammonium carbamyl phosphate, and dilithium carbamyl phosphate; and 5'-nucleotide such as 5'-purine ribotide, 5'-inosinic acid, 5'-guanylic acid, 5'-adenylic acid, 5'-xanthylic acid, 5'-uridylic acid, and 5'-cytidylic acid.

(3) Optimum pH: 5.2 (transphosphorylation reaction), 6.5 (phosphate ester hydrolytic reaction).

(4) pH stability: pH 3.5 to 12.0 (treatment at 30 °C for 60 minutes).

(5) Optimum temperature: about 35 °C.

(6) Temperature stability: stable up to 40 °C (treatment at pH 7.0 for 30 minutes).

(7) Effect of the addition of metal ion and inhibitor: This enzyme exhibits no activation phenomenon relevant to its activity by addition of any metal ion The activity is inhibited by Fe^{2+}, Ag^{2+}, Pb^{2+}, Hg^{2+}, and Cu^{2+}. The activity is also inhibited by iodoacetic acid.

(8) Molecular weight: A calculated molecular weight is about 188,000 in accordance with high-performance liquid chromatography (TSKgel G-3000SW, produced by Toyo Soda).

(9) Subunit molecular weight: A calculated subunit molecular weight is about 24,500 in accordance with SDS-polyacrylamide gel electrophoresis.

This enzyme also exhibits not only the activity to transfer phosphate group to nucleoside, but also the activity to

reversely hydrolyz phosphate ester, in th same mann r as the enzyme purified from th cell-free xtract of Morganella morganii NCIMB 10466. Moreover, this enzyme exhibits the phosphate ester hydrolytic activity (phosphomonoesterase activity) which is higher than th transphosphorylation activity by not less than 30 times. Accordingly, it has been clarified that this enzyme is an acid phosphatas .

Sodium pyrophosphate (15 g/dl) and inosine (3 g/dl) were dissolved in sodium acetate buffers each having pH of 5.5, 5.0, 4.5, 4.0, and 3.5, to which the enzyme sample described above was added so that a concentration of 50 units/dl was obtained. The reaction mixture was incubated at 30 °C for 6 hours while maintaining each pH, and the amount of produced 5'-inosinic acid was measured along with passage of time. Produced inosinic acid contained only 5'-inosinic acid. By-production of 2'-inosinic acid and 3'-inosinic acid was not observed at all. A result is shown in Fig. 2. The v locity of 5'-inosinic acid production was maximum at pH 5.0. However, the maximum accumulated amount of 5'-inosinic acid was higher at lower pH. The reaction condition at pH 4.0 was most efficient for production of 5'-inosinic acid. 5'-Inosinic acid was produced and accumulated in an amount of 1.56 g/dl by performing the reaction at 30 °C at pH 4.0 for 3 hours.

Example 5: Phosphorylation Reaction of Various Nucleosides by Acid Phosphatase Sample Derived from Escherichia blattae

Sodium pyrophosphate (15 g/dl) and inosine, guanosine, uridine, or cytidine (3 g/dl) were dissolved in sodium acetate buffer (pH 4.0), to which the enzyme sample prepared in Example 4 was added so that its concentration was 50 units/dl. The reaction mixture was incubated at 35 °C for 3 hours while maintaining pH at 4.0. The amount of produced nucleoside-5'-ester is shown in Table 5.

Produced nucleotide contained only nucleoside-5'-ester. By-production of nucleoside-2'-ester and nucleoside-3'-ester was not observed at all.

Table 5

Nucleoside	Product	Produced amount (g/dl)
Inosine	5'-inosinic acid	1.56
Guanosine	5'-guanylic acid	1.05
Uridine	5'-uridylic acid	1.87
Cytidine	5'-cytidylic acid	1.22

Example 6: Production of 5'-Inosinic acid from Various Phosphate Compounds as Phosphate Group Donors by Acid Phosphatase Sample Derived from Escherichia blattae

Inosine (2 g/dl) and sodium tripolyphosphate, sodium polyphosphate (trade name: Polygon P, produced by Chiyoda Chemical), disodium phenylphosphate, or disodium carbamyl phosphate (10 g/dl) as a phosphate group donor were dissolved in sodium acetate buffer (pH 4.0), to which the enzyme sample prepared in Example 4 was added so that its concentration was 50 units/dl. The reaction miture was incubated at 35 °C for 3 hours while maintaining pH at 4.0. The amount of produced 5'-inosinic acid is shown in Table 6.

5'-Inosinic acid was efficiently produced and accumulated by using any of the phosphate group donors. However, the accumulated amount of 5'-inosinic acid was the highest when sodium polyphosphate was used as the phosphate group donor.

Table 6

Phosphate group donor	Produced 5'-inosinic acid (g/dl)
Sodium tripolyphosphate	1.20
Sodium polyphosphate	1.79
Disodium phenylphosphate	1.50
Disodium carbamyl phosphate	1.53

Example 7: Isolation of Gene Coding for Acid Phosphatase from Chromosome of Morganella morganii

(1) D termination of N-terminal amino acid sequ nce

The acid phosphatase purified from th cell-free extract of Morganella morganii NCIMB 10466 in accordance with

the method described in Example 1 was adsorbed to DITC membrane (produced by Milligen/Biosearch), and its N-terminal amino acid sequence was determined by using Prosequencer 6625 (produced by Milligen/Biosearch). An N-terminal amino acid sequence comprising 20 residues shown in SEQ ID NO: 1 in Sequ nce Listing was determined.

(2) Isolation of DNA fragment containing gene coding for acid phosphatase

Chromosomal DNA was extracted from cultivated microbial cells of Morganella morganii NCIMB 10466 in accordance with a method of Murray and Thomson (Nucl. Acid Res., 4321, 8 (1980)). The chromosomal DNA was partially degraded with restriction enzyme Sau3AI. After that, DNA fragments of 3 to 6 kbp were fractionated by means of sucrose density gradient centrifugation. A plasmid vector pUC118 (produced by Takara Shuzo) was digested with r striction enzyme BamHI, which was ligated with the partially degraded chromosomal DNA fragments. DNA ligation was performed by using DNA ligation kit (produced by Takara Shuzo) in accordance with a designated method. After that, Escherichia coli JM109 (produced by Takara Shuzo) was transformed with an obtained DNA mixture in accordance with an ordinary method. Transformants were plated on an L agar medium containing 100 μg/ml of ampicillin, and they were grown to prepare a gene library.

A reaction solution containing 4 mM p-nitrophenylphosphoric acid and 100 mM MES/NaOH buffer (pH 6.5) was poured onto a surface of the agar medium on which the transformants had grown, and the temperature was kept at 30 °C for 15 minutes. Strains which had expressed the phosphatase activity liberated p-nitrophenol and exhibited a yellow color. Accordingly, transformants were selected by using this phenomenon as an index. As a result of screening for a gene expression library comprising about 20,000 strains of transformants, 30 strains of transformants which had expressed the phosphatase activity were obtained.

The transformants (30 strains), which had expressed the phosphatase activity, were subjected to single colony isolation. Single colonies were inoculated to an L-medium (2.5 ml) containing 100 μg/ml of ampicillin, and they were cultivated at 37 °C for 16 hours. Sodium acetate buffer (100 mM, pH 5.0; 50 μl) containing inosine (2 g/dl) and sodium pyrophosphate (10 g/dl) was added to microbial cells harvested from culture, and the reaction mixture was incubated at 30 °C for 16 hours. Production of 5'-inosinic acid was detected by HPLC analysis to select microbial strains having the transphosphorylation activity. As a result, we succeeded in obtaining 5 strains of transformants which exhibited the transphosphorylation activity and which were assumed to harbor a DNA fragment containing the objective acid phosphatase gene.

Example 8: Determination of Nucleotide Sequence of Acid Phosphatase Gene Derived from Morganella morganii NCIMB 10466

The inserted DNA fragment was analyzed by preparing a plasmid in accordance with an alkaline lysis method (Molecular Cloning 2nd edition (J. Sambrook, E. F. Fritsch and T. Maniatis, Cold Spring Harbour Laboratory Press, pl. 25 (1989)) from one strain of the transformants which were assumed to harbor the DNA fragment containing the acid phosphatase gene derived from Morganella morganii NCIMB 10466 obtained in Example 7. This plasmid was designated as pMPI501. Fig. 3 shows a determined restriction enzyme map of the inserted DNA fragment.

The region of the acid phosphatase gene was further specified by subcloning. As a result, it was suggested that this acid phosphatase gene was contained in a fragment having a size of 1.2 Kbp excised by restriction enzymes HindIII and EcoRI. Thus in order to determine the nucleotide sequence, plasmid DNA was constructed in which the fragment of 1.2 kbp was ligated with pUC118 having been digested with HindIII and EcoRI. Escherichia coli JM109 (produced by Takara Shuzo) was transformed with this plasmid DNA designated as pMPI505 in accordance with an ordinary method, which was plated on an L agar medium containing 100 μg/ml of ampicillin to obtain a transformant.

The plasmid was extracted in accordance with the alkaline lysis method from the transformant of Escherichia coli JM109 (produced by Takara Shuzo) harboring pMPI505 to determine the nucleotide sequence. The nucleotide sequence was determined in accordance with a method of Sanger (J. Mol. Biol., 143, 161 (1980)) by using Taq DyeDeoxy T rminator Cycle Sequencing Kit (produced by Applied Biochemical). A nucleotide sequence of a determined open reading frame is shown in SEQ ID NO: 2 in Sequence Listing. An amino acid sequence of the protein deduced from the nucleotide sequence is shown in SEQ ID NO: 3 in Sequence Listing. A partial sequence, which was completely coincident with the N-terminal amino acid sequence of the purified enzyme, was found in the amino acid sequence. The N-terminal of the purified enzyme starts from the 21th alanine residue of the sequence shown in SEQ ID NO: 3. Accordingly, it is assumed that the amino acid sequence shown in SEQ ID NO: 3 is that of a precursor protein, and that a peptide comprising a range from the 1st methionine residue to the 20th alanine residue is eliminated after translation. An amino acid sequence of a mature protein thus deduced is shown in SEQ ID NO: 4 in Sequence Listing. A molecular weight of the mature protein estimated from the amino acid sequence is calculated to be 24.9 kilodaltons, which is well coincident with th result of SDS-PAGE for the purified enzyme. According to the results described above, and becaus f the fact that the transformant harboring the plasmid containing this fragment exhibited th transphos-

phorylation activity, it was identified that this open reading frame was th r gion coding for the objectiv acid phosphatase.

The nucleotide sequence and the amino acid sequence w r resp ctively compar d with known sequences for homology. Data bases of EMBL and SWISS-PROT were used. As a result, it has b en revealed that the nucleotide sequence shown in SEQ ID NO: 2 in Sequence Listing is coincident with a nucleotide sequence of a known acid phosphatase gene derived from Morganella morganii (Thaller, M. C. et al., Microbiology, 140, 1341 (1994)) except that 54th G is A, 72th G is A, 276th T is G, 378th T is C, 420th G is T, 525th C is G, 529th C is T, and 531th G is A in the latter, and that the amino acid sequence shown in SEQ ID NO: 4 in Sequence Listing is the same as that of the acid phosphatase gene derived from Morganella morganii. Namely, the gene, which codes for the protein comprising the amino acid sequence shown in SEQ ID NO: 4 in Sequence Listing, is the acid phosphatase gene of Morganella morganii NCIMB 10466.

A precursor protein comprises 249 amino acids, and a molecular weight of the protein deduced from its sequence is 27.0 kilodaltons.

The strain of Escherichia coli JM109 transformed by a plasmid pMPI505, has been designated as AJ13143, which has been internationally deposited on February 23, 1996 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the provision of the Budapest Treaty, and awarded a deposition number of FERM BP-5422.

Example 9: Amplification of Activity by Expressing Gene of Acid Phosphatase Derived from Morganella morganii NCIMB 10466

Escherichia coli JM109/pMPI505 constructed in Example 8 was inoculated to an L-medium (50 ml) containing 100 μg/ml of ampicillin and 1 mM of IPTG, and it was cultivated at 37 °C for 16 hours. Microbial cells were harvested from its culture by centrifugation, and they were washed once with physiological saline. The microbial cells were suspended in 100 mM potassium phosphate buffer (5 ml, pH 7.2), and they were disrupted by means of a ultrasonic treatment p rformed at 4 °C for 20 minutes. The treated solution was centrifuged to remove an insoluble fraction, and thus,a cell-free extract was prepared.

The transphosphorylation activity of the obtained cell-free extract was measured while using controls of cell-free xtracts prepared from the wild type strain of Morganella morganii and Escherichia coli JM109 transformed with the plasmid pUC118 in the same manner as described above. A result is shown in Table 7. The transphosphorylation activity was not detected in Escherichia coli JM109/pUC118. The transphosphorylation activity was also low in the wild type strain of Morganella morganii. On the other hand, Escherichia coli JM109/pMPI505 exhibited a high transphosphorylation activity which was 150 times as high as that of the wild type strain of Morganella morganii in specific activity. According to the result, it has been demonstrated that the introduced DNA fragment allows Escherichia coli to express the acid phosphatase at a high level.

Table 7

Microbial strain	Transphosphorylation Activity (units/mg)
Morganella morganii NCIMB 10466	0.008
Escherichia coli JM109/pUC118	not detected
Escherichia coli JM109/pMPI505	1.250

Example 11: Isolation of Gene Coding for Acid Phosphatase from Chromosome of Escherichia blattae

(1) Determination of N-terminal amino acid sequence

The acid phosphatase purified from the cell-free extract of Escherichia blattae JCM 1650 was adsorbed to DITC membrane (produced by Milligen/Biosearch), and its N-terminal amino acid sequence was determined by using Prosequencer 6625 (produced by Milligen/Biosearch). An N-terminal amino acid sequence comprising 15 residues shown in SEQ ID NO: 8 in Sequence Listing was determined.

(2) Isolation of DNA fragment containing gene coding for acid phosphatase

Chromosomal DNA was extracted from cultivated cells of Escherichia blatta JCM 1650 in accordance with a method of Murray and Thomson (Nucl. Acid Res., 4321, 8 (1980)). The chromosomal DNA was partially degraded with Sau3AI. After that, DNA fragments of 3 to 6 kbp were fractionated by means of sucrose density gradient centrifugation.

A plasmid vector pUC118 (produced by Takara Shuzo) was digested with BamHI, which was ligated with the partially degraded chromosomal DNA fragments. DNA ligation was performed by using DNA ligation kit (produced by Takara Shuzo) in accordance with a d signated method. After that, Esch richia coli JM109 (produced by Takara Shuzo) was transformed with an obtained DNA mixture in accordanc with an ordinary m thod. Transformants were plated on an L agar medium containing 100 μg/ml of ampicillin, and they were grown to prepare a gene library.

A reaction solution containing 4 mM p-nitrophenylphosphoric acid and 100 mM MES/NaOH buffer (pH 6.5) was poured onto a surface of the agar medium on which the transformants had grown, and the temperature was kept at 30 °C for 15 minutes. Strains which had expressed the phosphatase activity liberated p-nitrophenol and exhibited a y llow color. Accordingly, transformants were selected by using this phenomenon as an index. As a result of screening for a chromosomal gene expression library comprising about 8,000 strains of transformants, 14 strains of transformants which had expressed the phosphatase activity were obtained.

The transformants (14 strains), which had expressed the phosphatase activity, were subjected to single colony isolation. Single colonies were inoculated to an L-medium (2.5 ml) containing 100 μg/ml of ampicillin, and they were cultivated at 37 °C for 16 hours. Sodium acetate buffer (100 mM, pH 5.0, 50 μl) containing inosine (2 g/dl) and sodium pyrophosphate (10 g/dl) was added to microbial cells harvested from culture liquids to perform the reaction at 30 °C for 16 hours. Production of 5'-inosinic acid was detected by HPLC analysis to select strains having the transphosphorylation activity. As a result, we succeeded in obtaining 3 strains of transformants which exhibited the transphosphorylation activity and which were assumed to harbor a DNA fragment containing the objective acid phosphatase gene.

Example 12: Determination of Nucleotide Sequence of Acid Phosphatase Gene Derived from Escherichia blattae JCM 1650

The inserted DNA fragment was analyzed by extracting a plasmid in accordance with the alkaline lysis method from one strain of the transformants which were assumed to harbor the DNA fragment containing the acid phosphatase g ne derived from Escherichia blattae JCM 1650 obtained in Example 11. This plasmid was designated as pEPI301. Fig. 5 shows a determined restriction enzyme map of the inserted DNA fragment.

The region of the acid phosphatase gene was further specified by subcloning. As a result, it was suggested that this acid phosphatase gene was included in a fragment having a size of 2.4 Kbp excised by restriction enzymes ClaI and BamHI. Thus in order to determine the nucleotide sequence, plasmid DNA was constructed in which the fragment was ligated with pBluescript KS(+) (produced by Stratagene) having been digested with ClaI and BamHI. Escherichia coli JM109 (produced by Takara Shuzo) was transformed with the plasmid DNA designated as pEPI305 in accordance with an ordinary method, which was plated on an L agar medium containing 100 μg/ml of ampicillin to obtain a transformant.

The plasmid was extracted in accordance with the alkaline lysis method from the transformant of Escherichia coli JM109 (produced by Takara Shuzo) harboring pEPI305 to determine the nucleotide sequence. A nucleotide sequence of a determined open reading frame is shown in SEQ ID NO: 6 in Sequence Listing. An amino acid sequence of the protein deduced from the nucleotide sequence is shown in SEQ ID NO: 7 in Sequence Listing. A partial sequence, which was completely coincident with the N-terminal amino acid sequence of the purified enzyme, was found in the amino acid sequence. The N-terminal of the purified enzyme starts from the 19th leucine residue of the sequence shown in SEQ ID NO: 7. Accordingly, it is assumed that the amino acid sequence shown in SEQ ID NO: 7 is that of a precursor protein and that a peptide comprising a range from the 1st methionine residue to the 18th alanine residue is eliminated after translation. An amino acid sequence of a mature protein thus deduced is shown in SEQ ID NO: 8 in Sequence Listing. Accordingly, an estimated molecular weight of the mature protein is calculated to be 25.1 kilodaltons, which is well coincident with the result of SDS-PAGE for the purified enzyme. According to the results described above, and because of the fact that the transformant harboring the plasmid containing this fragment exhibited the transphosphorylation activity, it was identified that this open reading frame was the region coding for the objective acid phosphatase.

Namely, the gene, which codes for the protein comprising the amino acid sequence shown in SEQ ID NO: 8 in Sequence Listing, is the acid phosphatase gene of Escherichia blattae JCM.1650.

The nucleotide sequence and the amino acid sequence were respectively compared with known sequences for homology. Data bases of EMBL and SWISS-PROT were used. As a result, it has been revealed that the protein shown in SEQ ID NO: 8 and DNA coding for it are novel. A precursor protein encoded by this gene comprises 249 amino acids, and a molecular weight of the protein deduced from its sequence is 27.0 kilodaltons.

The amino acid sequence was compared with known sequences respectively for homology. As a result, this protein exhibited a high degre of homology with the acid phosphatase of Providencia stuartii (77.1 %) with th acid phosphatase of Morganella morganii in Example 8 (77.1 %), and with acid phosphatase of Salmonella typhimurium (44.3 %).

The strain of Escherichia coli JM109 transformed by a plasmid pEPI305, has been designat d as AJ13144, which has be n int rnationally deposited on February 23, 1996 in National Institute of Bioscience and Human Technology of

Agency of Industrial Science and Technology (postal code: 305, 1-3, Higashi 1-chom , Tsukuba-shi, Ibaraki-k n, Japan) under the provision of the Budapest Treaty, and awarded a deposition number of FERM BP-5423.

Example 13: Amplification of Activity by Expressing G ne of Acid Phosphatase Derived from Escherichia blattae JCM 1650

Escherichia coli JM109/pEPI305 constructed in Example 12 was inoculated to an L medium (50 ml) containing 100 µg/ml of ampicillin and 1 mM of IPTG, and it was cultivated at 37 °C for 16 hours. Microbial cells were harvested from its culture by centrifugation, and they were washed once with physiological saline. The microbial cells were sus- p nded in 100 mM potassium phosphate buffer (5 ml, pH 7.2), and were disrupted by means of a ultrasonic treatment performed at 4 °C for 20 minutes. The treated solution was centrifuged to remove an insoluble fraction, and thus a cell-free extract was prepared.

The transphosphorylation activity of the obtained cell-free extract was measured while using controls of cell-free xtracts prepared from the wild type strain of Escherichia blattae and Escherichia coli JM109 transformed with the plasmid pBluescript KS(+) in the same manner as described above. A result is shown in Table 8. The transphosphorylation activity was not detected in Escherichia coli JM109/pBluescript KS(+). The transphosphorylation activity was also low in the wild type strain of Escherichia blattae. On the other hand, Escherichia coli JM109/pEPI305 exhibited a high transphosphorylation activity which was 120 times as high as that of the wild type strain of Escherichia blattae in sepcific activity. According to the result, it has been demonstrated that the introduced DNA fragment allows Escherichia coli to express the acid phosphatase at a high level.

Table 8

Microbial strain	Transphosphorylation Activity (units/mg)
Escherichia blattae JCM 1650	0.002
Escherichia coli JM109/pBluescript KS(+)	not detected
Escherichia coli JM109/pEPI305	0.264

Example 14: Production of 5'-Inosinic Acid from Inosine by Using Strain Harboring Acid Phosphatase Gene Derived from Escherichia blattae JCM 1650

Sodium pyrophosphate (12 g/dl) and inosine (6 g/dl) were dissolved in 100 mM sodium acetate buffer (pH 4.0), to which the microbial cells of Escherichia coli JM109/pEPI305 described above were added to give a cell concentration of 200 mg/dl as converted into a dry weight of the microbial cells. The reaction mixture was incubated at 35 °C for 10 hours while maintaining pH at 4.0, and the amount of produced 5'-inosinic acid was measured along with passage of time. Produced inosinic acid contained only 5'-inosinic acid. By-production of 2'-inosinic acid and 3'-inosinic acid was not observed at all. A result is shown in Fig. 6. 5'-Inosinic acid was produced and accumulated extremely efficiently in a short period of time in the reaction to produce 5'-inosinic acid from pyrophosphate and inosine by using this microorganism.

Example 15: Preparation of A Gene Encoding An Acid Phosphatase with lowered Phosphomonoesterase acitivity

As described in Examples 13 and 14, the strain harboring the acid phosphatase gene derived from Escherichia blattae expresses a considerable amount of the acid phosphatase, and 5'-inosinic acid is produced and accumulated extremely efficiently in a short period of time in the reaction to produce 5'-inosinic acid from pyrophosphate and inosine by using this microorganism. However, it has been revealed that the accumulated amount of 5'-inosinic acid does not exceed a certain degree because produced 5'-inosinic acid undergoes degradation by the phosphomonoesterase activity possessed by the acid phosphatase itself. Thus the enzyme was intended to be improved by introducing mutation into the acid phosphatase gene derived from Escherichia blattae cloned in Example 11, in accordance with the site-directed mutagenesis method by using PCR.

Oligonucleotides MUT300, MUT310, and MUT320 shown in SEQ ID NOs: 9, 10, and 11 in Sequence Listing were synthesized respectively in accordance with the phosphoamidite method by using a DNA synthesizer (Model 394 produced by Applied Biosystems).

The plasmid pEPI305 (1 ng) as a template prepared in Example 12, M13 primer RV (produced by Takara Shuzo) and MUT310 oligonucleotide (each 2.5 µmol) as primers, and Taq DNA polym rase (2.5 units, produced by Takara Shuzo) were added to 100 mM Tris-HCl buffer (pH 8.3, 100 µl) containing dATP, dCTP, dGTP, dTTP (each 200 µM), potassium chloride (50 mM), and magnesium chloride (1.5 mM) to perform a PCR reaction in which a cycle comprising

periods of 30 seconds at 94 °C, 2 minut s at 55 °C, and 3 minutes at 72 °C was rep at d 25 times. Th PCR reaction was performed by using Thermal Cycler PJ2000 type (produced by Takara Shuzo). Also, a PCR reaction was performed in the same manner as described above by using plasmid pEPI305 (1 ng) as a temperate, and M13 primer M3 (produced by Takara Shuzo) and MUT300 oligonucl otide (each 2.5 μmol) as primers. Each of th reaction solutions was purified by gel filtration to remove the primers by using Micro spin column S-400 (produced by Pharmacia).

Each of the PCR reaction products (1 μl) was added to 100 mM Tris-HCl buffer (pH 8.3, 95 μl) containing dATP, dCTP, dGTP, dTTP (each 200 μM), potassium chloride (50 mM), and magnesium chloride (1.5 mM), and it was heated at 94 °C for 10 minutes, followed by cooling to 37 °C over 60 minutes. After that, the temperature was kept at 37 °C for 15 minutes to form a heteroduplex. Taq DNA polymerase (2.5 units) was added thereto to perform a reaction at 72 °C for 3 minutes so that the heteroduplex was completed. After that, M13 primer RV and M13 primer M3 (each 2.5 μmol) were added to this reaction solution to perform a PCR reaction in which a cycle comprising periods of 30 seconds at 94 °C, 2 minutes at 55 °C, and 3 minutes at 72 °C was repeated 10 times.

A product of the second PCR reaction was digested with ClaI and BamHI followed by phenol/chloroform extraction and ethanol precipitation. This DNA fragment was ligated with pBluescript KS(+) having been digested with ClaI and BamHI. Escherichia coli JM109 (produced by Takara Shuzo) was transformed with obtained plasmid DNA in accordance with an ordinary method, which was plated on an L agar medium containing 100 μg/ml of ampicillin to obtain a transformant.

The plasmid was extracted from the transformant in accordance with the alkaline lysis method to determine its nucleotide sequence, confirming that the objective nucleotide was substituted. Thus a mutant gene coding for a mutant phosphatase was prepared in which the 74th glycine residue (GGG) of the mature protein was substituted with an aspartic acid residue (G*A*T). The plasmid containing this mutant gene was designated as pEPI310.

A mutant gene coding for a mutant phosphatase was prepared in which the 153th isoleucine residue (ATC) of the mature protein was substituted with a threonine residue (A*CC), in accordance with the same procedure as described above by using pEPI305 as a template, and MUT300 and MUT320 oligonucleotides as primers. The plasmid containing this mutant gene was designated as pEPI320. Further, a mutant gene coding for a mutant phosphatase was prepared in which the 74th glycine residue (GGG) of the mature protein was substituted with an aspartic acid residue (G*A*T), and the 153th isoleucine residue (ATC) of the mature protein was substituted with a threonine residue (A*CC), in accordance with the same procedure as described above by using pEPI310 as a template, and MUT300 and MUT320 oligonucleotides as primers. The plasmid containing this mutant gene was designated as pEPI330.

Escherichia coli JM109/pEPI310, Escherichia coli JM109/pEPI320, and Escherichia coli JM109/pEPI330 into which the plasmids containing the respective mutant acid phosphatase genes had been introduced, and Escherichia coli JM109/pEPI305 into which the plasmid containing the wild type acid phosphatase gene had been introduced were inoculated to an L medium (50 ml) containing 100 μg/ml of ampicillin and 1 mM of IPTG, and they were cultivated at 37 °C for 16 hours. Microbial cells were harvested from their culture, and they were washed once with physiological saline. The microbial cells were suspended in 100 mM potassium phosphate buffer (5 ml, pH 7.0), and they were disrupted by means of a ultrasonic treatment performed at 4 °C for 20 minutes. The treated solutions were centrifuged to remove insoluble fractions, and thus cell-free extracts were prepared. Phosphomonoesterase activities and transphosphorylation acitivities of the obtained cell-free extracts were measured at pH 4.0, and they were compared with an activity of the wild strain.

Table 9 shows the result of measurement of phosphomonoesterase activities and transphosphorylation acitivities of wild type acid phosphatase and acid phosphatases with lowered phosphomonoesterase activity. It shows that both of phosphomonoesterase activities and transphosphorylation acitivities of acid phosphatases with lowered phosphomonoesterase activity are lowered as compared with wild type acid phosphatase, and that degrees of decrease of phosphomonoesterase activities are larger than that of transphosphorylation activities, with the result that a ratio of phosphomonoesterase activity to transphosphorylation activity of the mutant acid phosphatase is lowered in comparison with the wild type acid phosphatase.

Table 9

Plasmid	Phosphomonoesterase activity (units/mg)	Transphosphorylation activity (units/mg)	Ratio [1)] (Relative value)
pEPI305	2.38	0.132	18.03 (100)

1): Ratio of phosphomonoesterase activities to the activities to produce nucleoside-5'-phosphate ester

Table 9 (continued)

Plasmid	Phosphomonoesterase activity (units/mg)	Transphosphorylation activity (units/mg)	Ratio [1] (Relative value)
pEPI310	0.26	0.019	13.68(76)
pEPI320	0.88	0.123	7.15(39)
pEPI330	0.42	0.070	6.00(33)

1): Ratio of phosphomonoesterase activities to the activities to produce nucleoside-5'-phosphate ester

Example 16: Production of 5'-Inosinic Acid from Inosine by Using The Strains Horboring A Gene Encoding The Acid Phosphatase with Lowered Phosphomonoesterase Activity

Escherichia coli JM109/pEPI310, Escherichia coli JM109/pEPI320, and Escherichia coli JM109/pEPI330 into which the plasmids containing the genes encoding the acid phosphatases with lowered phosphomonoesterase activity had been introduced, and Escherichia coli JM109/pEPI305 into which the plasmid containing the wild type acid phosphatase gene had been introduced were inoculated to an L medium (50 ml) containing 100 µg/ml of ampicillin and 1 mM of IPTG, and they were cultivated at 37 °C for 16 hours.

Sodium pyrophosphate (12 g/dl) and inosine (6 g/dl) were dissolved in sodium acetate buffer (pH 4.0), to which microbial cells of each of the strains of Escherichia coli obtained by the cultivation described above were added to give a cell concentration of 200 mg/dl as converted into a dry weight of the microbial cells. The reaction mixture was incubated at 35 °C for 32 hours while maintaining pH at 4.0, and the amount of produced 5'-inosinic acid was measured along with passage of time. A result is shown in Fig. 8.

In Fig. 7, the axis of ordinate indicates the concentration of 5'-inosinic acid (mg/dl), and the axis of abscissa indicates the reaction time (h). Progress of the reaction is indicated by solid circles for Escherichia coli JM109/pEPI305, solid triangles for Escherichia coli JM109/pEPI310, blanked circles for Escherichia coli JM109/pEPI320, and blanked squares for Escherichia coli JM109/pEPI330, as measured by using the cells of the respective strains.

The velocity of degradation of produced 5'-inosinic acid was decreased in the reaction to produce 5'-inosinic acid from inosine by using the stains harboring the acid phosphatase with lowered phosphomonoesterase activity. As a r sult, the yield and the accumulated amount of 5'-inosinic acid were increased. The highest accumulation of 5'-inosinic acid was exhibited by Escherichia coli JM109/pEPI330 as the strain harboring the gene encoding the acid phosphatase with lowered phosphomonoesterase activity in which the 74th glycine residue and the 153th isoleucine residue were substituted with the aspartic acid residue and the threonine residue respectively.

Example 17: Production of Various Nucleoside-5'-Phosphate Esters by Using The Strains Harboring A Gene Encoding The Acid Phosphatase with Lowered Phosphomonoesterase Activity

Escherichia coli JM109/pEPI330 into which the plasmid containing the gene encoding the acid phosphatase with lowered phosphomonoesterase activity had been introduced was inoculated to an L medium (50 ml) containing 100 µg/ml of ampicillin and 1 mM of IPTG, and it was cultivated at 37 °C for 16 hours.

Sodium pyrophosphate (12 g/dl), and inosine, guanosine, uridine, or cytidine (6 g/dl) as a phosphate group acceptor were dissolved in 100 mM sodium acetate buffer (pH 4.0), to which the microbial cells described above were added to give a cell concentration of 200 mg/dl as converted into a dry weight of the cells. The reaction mixture was incubated at 35 °C for 32 hours while maintaining pH at 4.0. Amounts of produced nucleoside-5'-phosphate esters are shown in Table 10. Produced nucleotide contained only nucleoside-5'-phosphate ester. By-production of nucleoside-2'-phosphate ester and nucleoside-3'-phosphate ester was not observed at all.

Table 10

Nucleoside	Product	Produced amount (g/dl)
Inosine	5'-inosinic acid	7.45
Guanosine	5'-guanylic acid	4.77
Uridine	5'-uridylic acid	8.93
Cytidine	5'-cytidylic acid	6.60

Example 18: Production of 5'-Inosinic Acid from Various Phosphate Compounds as Phosphate Group Donors by Using The Straing Harboring A Gene Encoding The Acid Phosphatase with Lower d Phosphomonoesterase Activity

Escherichia coli JM109/pEPI330 into which th plasmid containing the mutant acid phosphatase gene had been introduced was inoculated to an L medium (50 ml) containing 100 µg/ml of ampicillin and 1 mM of IPTG, and it was cultivated at 37 °C for 16 hours.

Inosine (6 g/dl) and sodium tripolyphosphat , sodium polyphosphat (trade name: Polygon P, produced by Chiyoda Chemical), disodium phenylphosphate, or disodium carbamyl phosphate (12 g/dl) as a phosphate group donor were dissolved in 100 mM sodium acetate buffer (pH 4.0), to which the microbial cells described above were added to give a cell concentration of 200 mg/dl as converted into a dry weight of the cells. The reaction mixture was incubated at 35 °C for 32 hours while maintaining pH at 4.0. The amount of produced 5'-inosinic acid is shown in Table 11. 5'-Inosinic acid was efficiently produced and accumulated by using any of the phosphate group donors. However, the accumulated amount of 5'-inosinic acid was the highest when polyphosphoric acid was used as the phosphate group donor.

Table 11

Phosphate group donor	Produced 5'-inosinic acid (g/dl)
Sodium tripolyphosphate	5.96
Sodium polyphosphate	8.84
Disodium phenylphosphate	7.60
Disodium carbamyl phosphate	7.73

Example 19

Studies on production of a new mutant acid phosphatase gene derived from E. blattae JCM1650 and enzymological properties of the mutant acid phosphatase gene:

In Examples 19 to 22, the transphosphorylation activity to a nucleoside was measured under the following conditions. The reaction was conducted at 30°C and a pH of 4.0 for 10 minutes using 1 ml of a reaction solution containing 40 µmol/ml of inosine, 100 µmol/ml of sodium pyrophosphate, 100 µmol/ml of a sodium acetate buffer (pH 4.0) and an nzyme. This reaction was terminated with the addition of 200 µl of 2-N hydrochloric acid. Then, the precipitate was removed through centrifugation, and the amount of 5'-inosinic acid formed through the transphosphorylation was determined under the above-mentioned conditions. The amount of the enzyme with which to produce 1 µmol of inosinic acid under these standard reaction conditions was defined as 1 unit.

Further, the transphosphorylation activity was measured by changing the inosine concentration from 10 to 100 µmol/ml under the reaction conditions of the above-mentioned composition, and the rate constant of inosine in the transphosphorylation activity was determined using the Hanes-Woolf plot [Biochem. J., 26, 1406 (1932)].

As described later, the detailed analysis was conducted with respect to the mutant enzyme by which to improve the productivity of the nucleoside-5'-phosphate ester described in Example 15. Consequently, it was found that the affinity for nucleoside of the mutant enzyme was improved by 2 times as compared with that of the wild-type enzyme. Therefore, the present inventors considered that the productivity of the nucleoside-5'-phosphate ester would be improved by increasing the affinity for nucleoside of the above-mentioned enzyme, and they further modified the enzyme by the genetic engineering method.

Plasmid pEPI305 containing the gene encoding the wild-type acid phosphatase derived from E. blattae described in Example 15 was used, and the site specific mutation was introduced into this plasmid DNA by the genetic engineering method to produce a gene encoding the mutant acid phosphatase. pEPI305 is a plasmid DNA formed by binding a DNA fragment of 2.4 Kbp cleaved with restriction endonucleases ClaI and BamHI and containing a gene encoding a wild-type acid phosphatase derived from E. blattae JCM1650 to pBluescript KS(+) (supplied by Stratagene) cleaved with ClaI and BamHI. The base sequence of the gene encoding the acid phosphatase is represented by SEQ ID NO: 6 in Sequence Listing. Further, an amino acid sequence of a precursor protein anticipated from this base sequence is r presented by SEQ ID NO: 7 in Sequence Listing. From the analytical results of the purified enzyme (described in Example 4), the amino acid sequence of the maturation protein is presumed to be represented by SEQ ID NO: 8 in Sequence Listing.

Oligonucleotides MUT300 (SEQ ID NO: 9 in Sequence Listing), MUT310 (SEQ ID NO: 10 in Sequence Listing), MUT320 (SEQ ID NO: 11 in Sequence Listing), MUT330 (SEQ ID NO: 12 in Sequence Listing), MUT340 (SEQ ID NO: 13 in Sequence Listing), MUT350 (SEQ ID NO: 14 in Sequence Listing), MUT360 (SEQ ID NO: 15 in Sequence Listing), MUT370 (SEQ ID NO: 16 in Sequence Listing), MUT380 (SEQ ID NO: 17 in Sequence Listing) and MUT390 (SEQ ID NO: 18 in Sequence Listing) having the sequences shown in Sequence Listing were synthesized by the phosphoamidite method using a DNA synthesizer (Model 394 supplied by Applied Biosyst m).

One nanogram of pEPI305 as a template, 2.5 µmols of M13 Primer RV (supplied by Takara Shuzo Co., Ltd.), 2.5

μmols of oligonucl otide MUT310 and 2.5 units of tac DNA polymerase (supplied by Takara Shuzo Co., Ltd.) were added to 100 μl of a Tris-hydrochloride buffer (pH 8.3) containing 200 μM of dATP, 200 μM of dCTP, 200 μM of dGTP, 200 μM of dTTP, 50 mM of potassium chloride and 1.5 mM of magnesium chloride. PCR was conducted in which a three-part step, namely, at 94°C for 30 seconds, at 55°C for 2 minutes and at 72°C for 3 minutes was repeated 25 times. In this reaction, a thermal cycler PJ2000 model (supplied by Takara Shuzo Co., Ltd.) was used. Separately, PCR was likewise conducted using 1 ng of plasmid DNA pEPI305 as a template, 2.5 μmols of M13 Primer M3 (supplied by Takara Shuzo Co., Ltd.) as a primer and 2.5 μmols of oligonucleotide MUT300. Each of the reaction solutions was purified through gel filtration using a microspin column S-400 (supplied by Pharmacia) to remove the primer.

One microliter of each of the PCR solutions was added to 95 μl of a 100-mM Tris-hydrochloride buffer (pH 8.3) containing 200 μM of dATP, 200 μM of dCTP, 200 μM of dGTP, 200 μM of dTTP, 50 mM of potassium chloride and 1.5 mM of magnesium chloride. The mixture was heated at 94°C for 10 minutes, then cooled to 37°C over the course of 60 minutes, and warmed at 37°C for 15 minutes to form a heteroduplex. To this were added 2.5 units of tac DNA polymerase, and the reaction was conducted at 72°C for 3 minutes to complete the heteroduplex. Subsequently, 2.5 μmols of M13 Primer RV and 2.5 μmols of M13 Primer M3 were added to the reaction solution, and PCR was conducted in which a three-part step, namely, at 94°C for 30 seconds, at 55°C for 2 minutes and at 72°C for 3 minutes was repeated 10 times.

The second PCR product was cleaved with ClaI and BamHI, then extracted with a mixture of phenol and chloroform, and precipitated with ethanol. This DNA fragment was bound to pBluescript KS (+) cleaved with ClaI and BamHI. E. coli JM109 (supplied by Takara Shuzo Co., Ltd.) was transformed in a usual manner using the resulting plasmid DNA. This was plated on an L agar medium containing 100 μg/ml of ampicillin to obtain a transformant. A plasmid was prepared from the transformant by an alkali bacteriolysis method, the base sequence was determined, and it was identified that the desired base was substituted. The determination of the base sequence was conducted by the method of Sanger et al. [J. Mol. Biol., 143, 161 (1980)] using a Taq DyeDeoxy Terminator Cycle Sequencing Kit (supplied by Applied Biochemical). In this manner, the mutant gene encoding the mutant phosphatase in which the 74th glycine residue (GGG) of the maturation protein was substituted with the aspartic acid residue (G*A*T) was produced. This mutant gene-containing plasmid was designated pEPI310 (Example 15).

The above-mentioned procedure was repeated using the plasmid having the mutation introduced therein as a template to cumulatively introduce the site-specific mutation. A plasmid was produced from the transformant by the alkali bacteriolysis method, the base sequence was determined, and it was identified that the desired base was substituted. The resulting mutant genes encoding the mutant phosphatase and the mutation sites are shown in Table 12. The amino acid residue in the mutation site indicates an amino acid residue in the amino acid sequence of the mature protein represented by SEQ ID NO: 8 in Sequence Listing.

Table 12

plasmid name	starting material	primer	mutation position and substituted amino acid
pEPI305	-		wild type
pEPI310	pEPI305	MUT300	74Gly(GGG)→ASp(G*A*T)
		MUT310	
pEPI330	pEPI310	MUT300 MUT320	74Gly(GGG)→Asp(G*A*T) 153Ile(ATC)→Thr(A*CC)
pEPI340	pEPI330	MUT300 MUT330	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 74Gly(GGG)→Asp(G*A*T) 153Ile(ATC)→Thr(A*CC)
pEPI350	pEPI340	MUT300 MUT340	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 74Gly(GGG)→Asp(G*A*T) 85Ser(TCC)→Tvr(T*AC) 153Ile(ATC)→Thr(A*CC)
pEPI360	pEPI340	MUT300 MUT350	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG)

Table 12 (continued)

plasmid name	starting material	primer	mutation position and substituted amino acid
			66Glu(GAA)→Ala(G*CA) 74Gly(GGG)→Asp(G*A*T) 135Thr(ACC)→Lys(A*A*A) 136Glu(GAG)→Asp(GA*C) 153Ile(ATC)→(A*CC)
pEPI370	pEPI360	MUT300 MUT360	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 69Asn(AAC)→Asp(*GAC) 71Ser(AGC)→Ala(*G*CC) 72Ser(AGT)→Ala(*G*CT) 74Gly(GGG)→Asp(G*A*T) 135Thr(ACC)→Lys(A*A*A) 136Glu(GAG)→Asp(GA*C) 153Ile(ATC)→(A*CC)
pEPI380	pEPI370	MUT300 MUT370	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 69Asn(AAC)→Asp(*GAC) 71Ser(AGC)→Ala(*G*CC) 72Ser(AGT)→Ala(*G*CT) 74Gly(GGG)→Asp(G*A*T) 116Asp(GAT)→Glu(GA*A) 135Thr(ACC)→Lys(A*A*A) 136Glu(GAG)→Asp(GA*C) 153Ile(ATC)→(A*CC)
pEPI390	pEPI380	MUT300 MUT380	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 69Asn(AAC)→Asp(*GAC) 71Ser(AGG)→Ala(*G*CC) 72Ser(AGT)→Ala(*G*CT) 74Gly(GGG)→Asp(G*A*T) 116Asp(GAT)→Glu(GA*A) 130Ser(TCT)→Glu(*G*A*A) 135Thr(ACC)→Lys(A*A*A) 136Gln(GAG)→Asp(GA*C) 153Ile(ATC)→Thr(A*CC)
pEPI400	pEPI380	MUT300 MUT390	63Leu(CTG)→Gln(C*AG) 65Ala(GCG)→Gln(*C*AG) 66Glu(GAA)→Ala(G*CA) 69Asn(AAC)→Asp(*GAC) 71Ser(AGC)→Ala(*G*CC) 72Ser(AGT)→AlN(G*A*T) 74Gly(GGG)→Asp(G*A*T) 92Ala(GCC)→Ser(*A*GC) 94Ala(GCg)→Glu(G*A*A) 116Asp(GAT)→Glu(GA*A)

Table 12 (continued)

plasmid name	starting material	primer	mutation position and substituted amino acid
			135Thr(ACC)→Lys(A*A*) 136Glu(GAG)→(GA*C) 153Ile(ATC)→Thr(A*CC)

Each of E. coli JM109/pEPI330, E. coli JM109/pEPI340, E. coli JM109/pEPI350, E. coli JM109/pEPI360, E. coli JM109/pEPI370, E. coli JM109/pEPI380, E. coli JM109/pEPI390 and E. coli JM109/pEPI400 each having introduced therein a plasmid containing the mutant acid phosphatase gene and E. coli JM109/pEPI305 having introduced therein a plasmid containing a wild-type acid phosphatase gene was inoculated into 50 ml of an L medium containing 100 μg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours. The cells were collected from 2 liters of the culture solution of each of the strains through centrifugation, and washed once with a physiological saline solution. The cells were suspended in 50 ml of a 100-mM phosphate buffer (pH 7.0), and sonicated at 4°C for 20 minutes to disrupt the cells. The thus-treated solution was centrifuged to remove insoluble fractions and prepare a cell-free extract. Each of the acid phosphatases was purified from each of the cell-free extracts by the method described in Example 4. Each of the enzyme products was uniform in the SDS-polyacrylamide electrophoresis.

The rate constant of inosine in the transphosphorylation of the purified mutant acid phosphatases and wild-type acid phosphatase was measured, and the results are shown in Table 13. It was found that the mutant enzyme expressed in E. coli JM109/pEPI330 having the improved productivity of the nucleoside-5'-phosphate ester as described in Example 15 has decreased Vmax but greatly decreased the Km value to inosine which means the increased affinity for inosine by twice or more as compared with the wild-type enzyme expressed in E. coli JM109/pEPI305. This suggested that the productivity of the nucleoside-5'-phosphate ester of this mutant enzyme was greatly improved not only because of the decrease in the nucleotidase activity but also because of the improvement in the affinity for nucleoside which was an important factor. Accordingly, it was expected that the increase in the affinity for nucleoside leads to the improvement in the productivity.

The new mutant enzymes expressed in E. coli JM109 having been introduced therein with the new mutant enzyme gene produced in this Example exhibited the affinity for inosine which was more improved than that of E. coli JM109/pEPI330 described in Example 15. Thus, it was expected that the productivity of the nucleoside-5'-phosphate ester was improved. Further, the mutant enzyme expressed in E. coli JM109/pEPI380 not only improved the affinity for inosine but also increased the Vmax value as compared with the wild-type enzyme. Still further, it was expected that the productivity of the nucleoside-5'-phosphate ester was improved.

Table 13

Strain of an enzyme	Km(mM)	Vmax(unit/mg)
E. coli JM109/pEPI305	202	1.83
E. coli JM109/pEPI330	109	1.39
E. coli JM109/pEPI340	85	1.03
E. coli JM109/pEPI350	85	0.93
E. coli JM109/pEPI360	55	1.33
E. coli JM109/pEPI370	42	1.15
E. coli JM109/pEPI380	42	2.60
E. coli JM109/pEPI390	42	2.58
E. coli JM109/pEPI400	43	2.11

Example 20

Production of 5'-inosinic acid using a new mutant acid phosphatase gene-containing strain:

Each of E. coli JM109/pEPI330, E. coli JM109/pEPI340, E. coli JM109/pEPI360, E. coli JM109/pEPI370 and E. coli JM109/pEPI380 each having introduced therein the plasmid containing the mutant acid phosphatase gene was inoculated into 50 ml of an L medium containing 100 μg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours.

Pyrophosphoric acid (15 g/dl) and 8 g/dl of inosine were dissolved in an acetate buffer (pH 4.0). To the solution was add d E. coli JM109 strain having introduc d th rein the above-mention d mutant and wild-type acid phosphatas genes such that the concentration reached 200 mg/dl in terms of the dry cell weight. The r action was conducted at

35°C for 32 hours while maintaining the pH at 4.0, and the amount of 5'-inosinic acid formed over the course of time was measured. Inosinic acid formed was only 5'-inosinic acid, and the formation of 2'-inosinic acid and 3'-inosinic acid as by-products was not observed at all. The results are shown in Fig. 8.

E. coli JM109/pEPI330 described in Example 15 showed the accumulation of 5'-inosinic acid in a large amount. Although the substrate still remained, the formation of 5'-inosinic acid stopped when the amount of 5'-inosinic acid accumulated reached 7.5 g/dl, and the amount of 5'-inosinic acid was no longer increased. By contrast, the new mutant acid phosphatase gene-containing strains provided the large amount of 5'-inosinic acid accumulated. Especially, in the reaction using E. coli JM109/pEPI370 and E. coli JM109/pEPI380, the larger amount of 5'-inosinic acid accumulated was provided. In addition, the reaction rate was high, showing that the productivity of 5'-inosinic acid was further improved greatly. In particular, in E. coli JM109/pEPI380, the reaction rate was high, and quite a high reactivity was shown.

Example 21

Production of various nucleoside-5'-phosphate esters using a new mutant acid phosphatase gene-containing strain:

E. coli JM109/pEPI380 having introduced therein the plasmid containing the new mutant acid phosphatase gene was inoculated into 50 ml of an L medium containing 100 μg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours.

Pyrophosphoric acid (15 g/dl) and 8 g/dl of inosine, guanine, uridine or cytidine as a phosphate acceptor were dissolved in a 100-mM acetate buffer (pH 4.5). To this was added the above-mentioned strain such that the concentration reached 100 mg/dl in terms of the dry cell weight. The reaction was conducted at 35°C for 12 hours while maintaining the pH at 4.0. The amount of the nucleoside-5'-phosphate ester formed is shown in Table 4. The phosphorylation proceeded well with any of the nucleosides to form and accumulate the corresponding nucleoside-5'-phosphate esters. The nucleotide formed was only the nucleoside-5'-phosphate ester, and the formation of a nucleoside-2'-phosphate ester and a nucleoside-3'-phosphate ester as by-products was not observed at all.

Table 14

Nucleoside	Product	Amount of the Product (g/dl)
inosine	5'-inosinic acid	12.05
guanosine	5'-guanylic acid	5.78
uridine	5'-uridylic acid	13.28
cytidine	5'-cytidylic acid	10.65

Example 22

Production of 5'-inosinic acid using a new acid phosphatase gene-containing strain and various phosphoric acid compounds as a phosphate donor:

E. coli JM109/pEPI380 having introduced therein the plasmid containing the new mutant acid phosphatase gene was inoculated into 50 ml of an L medium containing 100 μg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours.

Inosine (6 g/dl) and 15 g/dl of a tripolyphosphate, a polyphosphate ("Polygon P", a trade name for a product of Chiyoda Kagaku K.K.), disodium phenylacetate or disodium carbamylphosphate were dissolved in a 100-mM acetate buffer (pH 4.0). To this was added the above-mentioned strain such that the concentration reached 100 mg/dl in terms of the dry cell weight. The reaction was conducted at 35°C for 12 hours while maintaining the pH at 4.0. The amount of 5'-inosinic acid formed was shown in Table 15. 5'-Inosinic acid was formed and accumulated at good efficiency with any of the phosphate donors. Especially when a polyphosphate was used as a phosphate donor, 5'-inosinic acid was accumulated in the largest amount.

Table 15

Phosphate donor	Amount of 5'-inosinic acid formed (g/dl)
sodium tripolyphosphate	10.84
sodium polyphosphate	13.35
disodium phenylphosphate	12.84
disodium carbamylphosphate	12.42

Table 15 (continued)

Phosphate donor	Amount of 5'-inosinic acid formed (g/dl)
potassium lithium acetylphosphate	10.65

Example 23: Isolation of Acid Phosphatase Gene Derived from Chromosome of Providencia stuartii and Determination of Nucleotide Sequence of the Gene

Oligonucleotides, PRP1 and PRP2, having nucleotide sequences illustrated in SEQ ID NO: 19 and 20 in Sequence Listing, respectively, were synthesized. These oligonucleotides are designed to amplify a gene coding for acid phosphatase of Providencia stuartii on the basis of known nucleotide sequence of the gene coding for acid phosphatase of Providencia stuartii (Database of EMBL Accession number X64820).

Chromosomal DNA was extracted from cultivated microbial cells of Providencia stuartii ATCC 29851 in accordance with a method of Murray and Thomson (Nucl. Acid Res., 4321, 8 (1980)). The chromosomal DNA (0.1 ng) as a template, oligonucleotides PRP1 and PRP2 (each 2.5 µmol) as primers, and Taq DNA polymerase (2.5 units, produced by Takara Shuzo) were added to 100 mM Tris-HCl buffer (pH 8.3, 100 µl) containing dATP, dCTP, dGTP, dTTP (each 200 µM), potassium chloride (50 mM), and magnesium chloride (1.5 mM) to perform a PCR reaction in which a cycle comprising periods of 30 seconds at 94 °C, 2 minutes at 55 °C, and 3 minutes at 72 °C was repeated 30 times. The reaction solution was subjected to agarose gel electrophoresis, followed by recovering the amplified DNA fragment of about 1 kbp'by means of glass powders (made by Takara Shuzo). The gene fragment was digested with BamHI, which was ligated with pUC118 degiested with BamHI. The plasmid obtained as described above was designated as pPRP100.

Phosphomonoesterase activity and transphosphorylation activity of Escherichia coli JM109/pPRP100, a transformant to which pPRP100 was introduced, were mesured. As a result, the strain showed an activity to transphosphorylate to nucleoside as well as phosphomonoesterase activity.

The plasmid was extracted in accordance with the alkaline lysis method from the transformant of Escherichia coli JM109/pPRP100 to determine the nucleotide sequence. A nucleotide sequence of a determined open reading frame and an amino acid sequence of the protein deduced from the nucleotide sequence are shown in SEQ ID NO: 21 and 22 in Sequence Listing. The nucleotide sequence of the open reading frame is completely coincident with the nucleotide s quence of the known acid phosphatase gene of Providencia stuartii.

Example 24: Isolation of Acid Phosphatase Gene Derived from Chromosomes of Enterobacter aerogenes, Klebsiella planticola and Serratia ficaria and Determination of Nucleotide Sequences of the Genes

Chromosomal DNA was extracted from cultivated microbial cells of Enterobacter aerogenes IFO 12010, Klebsiella planticola IFO 14939 and Serratia ficaria IAM 13540 in accordance with a method of Murray and Thomson (Nucl. Acid Res., 4321, 8 (1980)). Then, in accordance with the method described in Example 7(2), a chromosomal gene expression library comprising about 20,000 transformants of Escherichia coli JM109 was constructed and screened to obtain transformants which showed transphosphorylation activity. It was considered that each of these transformants harbours the acid phosphatase gene derived from each of the original strains.

Plasmid DNA was extracted from one of the transformants of Escherichia coli which was considered to have the acid phosphatase gene derived from Enterobacter aerogenes IFO 12010 in accordance with an alkaline lysis method and the inserted DNA of the plasmid was analyzed. The above plasmid was designated as pENP100. A restriction enzyme map of the inserted DNA derived from Enterobacter aerogenes IFO 12010 is shown in Fig. 9.

As a result of specifying the region of acid phosphatase gene by subcloning, it was suggested that the acid phosphatase gene is contained in the 1.6 kbp fragment excised by restriction enzymes SalI and KpnI. Then, the SalI-KpnI fragment was ligated with pUC118 which was digested with SalI and KpnI to construct a plasmid. The resulting plasmid was designated as pENP110.

According to the procedure as described above, plasmid DNA was extracted from one of the transformants of Escherichia coli which was considered to have the acid phosphatase gene derived from Klebsiella planticola IFO 14939 in accordance with an alkaline lysis method and the insert DNA of the plasmid was analyzed. The above plasmid was designated as pKLP100. A restriction enzyme map of the inserted DNA derived from Klebsiella planticola IFO 14939 is shown in Fig. 10.

As a result of specifying the region of acid phosphatase gene by subcloning, it was suggested that the acid phosphatase gene is contained in the 2.2 kbp fragment excised by restriction enzymes KpnI andEcoRI. Then, the KpnI-EcoRI fragment was ligated with pUC118 which was digested with KpnI and EcoRI to construct a plasmid. The resulting plasmid was designated as pKLP110.

Similarly, plasmid DNA was extracted from one of the transformants of Escherichia coli which was considered to

have the acid phosphatase gene derived from S rratia ficaria IAM 13540 in accordance with an alkaline lysis method and the inserted DNA of the plasmid was analyzed. The above plasmid was designated as pSEP100. A restriction nzyme map of the inserted DNA d rived from Serratia ficaria IAM 13540 is shown in Fig. 11.

As a result of specifying the region of acid phosphatase gene by subcloning, it was suggested that the acid phosphatase gene is contained in the 1.4 kbp fragment excised by restriction enzymes HindIII. Then, the HindIII fragment was ligated with pUC118 which was digested with HindIII to construct a plasmid. The resulting plasmid was designated as pSEP110.

Then, the plasmid DNAs were extracted from the transformants, Escherichia coli JM109/pENP110, Escherichia coli JM109/pKLP110 and Escherichia coli JM109/pSEP110, to which pENP110 pKLP110 and pSEP110 had been introduced, respectively, in accordance with an alkaline lysis method. The nucleotide sequences of inserts of these plasmids were determined in accordance with the method described in Example 8. The determined nucleotide sequences of open reading frames of the inserts are shown in SEQ ID NO :23 for Enterobacter aerogenes IFO 12010, in SEQ ID NO: 25 for Klebsiella planticola IFO 14939 and in SEQ ID NO: 27 for Serratia ficaria IAM 13540. Additionally, the deduced amino acid sequences are shown in SEQ ID NOs: 24, 26 and 28, respectively. Because of the fact that the transformants harboring the plasmids containing these fragments exhibited the transphosphorylation activity, it was identified that these open reading frames were the objective acid phosphatase genes.

The nucleotide sequences and the deduced amino acid sequences were respectively compared with known sequences for homology. Data bases of EMBL and SWISS-PROT were used. As a result, it has been revealed that the genes illustrated in SEQ ID NO: 23, 25 and 27 in Sequence Listing are new genes. It is assumed that the protein encoded by the gene derived from Enterobacter aerogenes IFO 12010 comprises 248 amino acid residues, the protein encoded by the gene derived from Klebsiella planticola IFO 14939 comprises 248 amino acid residues and the protein encoded by the gene derived from Serratia ficaria IAM 13540 comprises 244 amino acid residues. There is a possibility that these proteins may be precursor proteins like the acid phosphatases derived from Morganella morganii and Escherichia blattae.

The amino acid sequences deduced from the nucleotide sequences are shown in Fig. 12 in one-letter together with the deduced amino acid sequence of the acid phosphatase derived from Morganella morganii NCIMB 10466 obtained in Example 8, that of Escherichia blattae JCM 1650 obtained in Example 12 and the known amino acid sequence of the acid phosphatase of Providencia stuartii (EMBL Accession number X64820). Common amino acid residues among all of the amino acids sequences are indicated with asterisks under the sequences in Fig. 12.

As shown in Fig. 12, the amino acid sequences of the acid phosphatases derived from six strains are highly homologous each other and 130 amino acid residues are common among all of the amino acid sequences. Thus, it is assumed that these acid phosphatases have similar functions.

Example 25: Amplification of Activity by Expressing Gene of Acid Phosphatase Derived from Enterobacter aerogenes, Klebsiella planticola and Serratia ficaria

Escherichia coli JM109/pPRP100 constructed in Example 23, Escherichia coli JM109/pENP110, Escherichia coli JM109/pKLP110 and Escherichia coli JM109/pSEP110 constructed in Example 24 were inoculated to an L-medium (50 ml) containing 100 µg/ml of ampicillin and 1 mM of IPTG, and were cultivated at 37 °C for 16 hours. Microbial cells were harvested from these cultures by centrifugation, and they were washed once with physiological saline. The microbial cells were suspended in 100 mM potassium phosphate buffer (5 ml, pH 7.0), and they were disrupted by means of a ultrasonic treatment performed at 4 °C for 20 minutes. The treated solutions were centrifuged to remove an insoluble fraction, and thus cell-free extracts were prepared.

The transphosphorylation activities of the obtained cell-free extracts were measured while using controls of cell-free extracts prepared from Providencia stuartii ATCC 29851, Enterobacter aerogenes IFO 12010, Klebsiella planticola IFO 14939, Serratia ficaria IAM 13450, and Escherichia coli JM109 transformed with the plasmid pUC118 in the same manner as described above. Results are shown in Table 16. The transphosphorylation activities were low in all of the wild type strains. The transphosphorylation activity was not detected in Escherichia coli JM109/pUC118. On the other hand, the transformants of Escherichia coli JM109 to which the acid phosphatase genes were introduced exhibited high transphosphorylation activities in comparison with wild type strains. According to the result, it has been demonstrated that each of the introduced DNA fragment allow Escherichia coli to express the acid phosphatase at a high level.

Table 16

Microbial strain	Transphosphrylation Activity (units/mg)
Providencia stuartii ATCC 29851	0.005
Enterobacter aerogenes IFO 12010	0.002

Table 16 (continued)

Microbial strain	Transphosphrylation Activity (units/mg)
Klebsiella planticola IFO 14939	0.002
Serratia ficaria IAM 13450	0.001
Escherichia coli JM109/pUC118	not detected
Escherichia coli JM109/pPRP100	0.833
Escherichia coli JM109/pENP110	0.301
Escherichia coli JM109/pKLP110	0.253
Escherichia coli JM109/pSEP110	0.123

Example 26

Production of a mutant acid phosphatase gene having an improved temperature stability:

As described in Examples 20, 21 and 22, the E. blattae-derived mutant acid phosphatase gene-containing strain produced in Example 19 expressed the considerable amount of the acid phosphatase. In the production of 5'-inosinic acid from pyrophosphoric acid and inosine using this strain, 5'-inosinic acid was formed and accumulated in the high conversion yield. The optimum reaction temperature of this acid phosphatase was 35°C. However, when this reaction was conducted at a higher temperature, the reaction rate was increased, and the reaction was conducted upon increasing the nucleoside concentration of the phosphate acceptor in the reaction solution. Accordingly, it was expected that the nucleoside-5'-phosphate ester could be produced more efficiently for a shorter period of time. Thus, the temperature stability of the enzyme was improved upon introducing the mutation into the E. blattae-derived acid phosphatase gene cloned in Example 19 by the site specific mutation method using PCR.

Plasmid pEPI380 containing the gene encoding the E. blattae JCM1650-derived mutant acid phosphatase described in Example 19 was used, and the site specific mutation was introduced into this plasmid DNA by the genetic ngineering method to produce the gene encoding the mutant acid phosphatase having the increased temperature stability. pEPI380 is a plasmid DNA obtained by binding a DNA fragment of 2.4 Kbp containing the gene encoding the mutant acid phosphatase derived from E. blattae JCM1650 and cleaved with restriction endonucleases C1aI and BamHI to pBluescript KS(+) (supplied by Stratagene) cleaved with C1aI and BamHI. The amino acid sequence of the mature protein anticipated from the base sequence of the gene encoding the acid phosphatase is presumed to be 11 amino acid resides shown in Table 12 in Example 19 in the sequence represented by SEQ ID NO: 8 in Sequence Listing.

Oligonucleotides MUT300 (SEQ ID NO: 9 in Sequence Listing), MUT400 (SEQ ID NO: 29 in Sequence Listing) and MUT410 (SEQ ID NO: 30 in Sequence Listing) having the sequences shown in Sequence Table were synthesized by the phosphoamidite method using a DNA synthesizer (Model 394 supplied by Applied Biosystem).

A mutant gene encoding a mutant phosphatase in which the 104th glutamic acid residue (GAG) of a maturation protein was substituted with a glycine residue (GG*T*) was produced by the method with PCR as in Example 15 using pEPI380 described in Example 19 as a template and MUT300 and MUT410 as primers for introduction of mutation. This mutant gene-containing plasmid was designated pEPI410. Likewise, a mutant gene encoding a mutant phosphatase in which the 151st threonine residue (ACC) was substituted with an alanine residue (G*CC) was produced using pEPI380 as a template and oligonucleotides MUT300, MUT310 and MUT420 as primers for introduction of mutation. This mutant gene-containing plasmid was designated pEPI420.

A plasmid was produced from the transformant of E. coli JM109 having introduced therein plasmids pEPI410 and pEPI420 containing the mutant phosphatase gene by the alkali bacteriolysis method, the base sequence was determined, and it was identified that the desired base was substituted.

Each of E. coli JM109/pEPI410 and E. coli JM109/pEPI420 having introduced therein the mutant acid phosphatase gene as produced in this Example and E. coli JM109/pEPI380 described in Example 19 was inoculated in 50 ml of an L medium containing 100 μg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours. The cells were collected from 50 ml of the culture solution of each of the strains, and washed once with a physiological saline solution. These cells were suspended in 5 ml of a 100-mM phosphate buffer (pH 7.0), and sonicated at 4°C for 20 minutes to mill the cells. The thus-treated solution was centrifuged to remove insoluble fractions and prepare a cell-free extract.

The cell-free extract formed from each of the strains was warmed at temperatures ranging from 0°C to 80°C with a pH of 7.0 for 30 minutes. After the completion of the warming, the transphosphorylation was conducted under the standard reaction conditions of pH of 4.0 and 30°C using the cell-fre xtracts treated at various t mperatures, and the residual activity was measured. The results are shown in Fig. 13. The mutant enzyme expressed in E. coli JM109/pEPI380 described in Example 19 was stable in the tr atment at 40°C for 30 minutes, but the decrease in th activity was observ d at higher temperatures. By contrast, the new mutant enzyme expressed in E. coli JM109/pEPI410

and E. coli JM109/pEPI420 having introduced ther in the new mutant enzyme gene as produc d in this Example improved the temperature stability, and the decrease in the activity was not observed even through the treatment at 50°C for 30 minutes. It was thus expected that when a nucl oside-5'-phosphate ester was produced using these strains at a high temperatur , the productivity was furth r improved.

Example 27

Production of 5'-inosinic acid and 5'-guanylic acid using a mutant acid phosphatase gene-containing strain having an improved temperature stability:

Each of E. coli JM109/pEPI410 and E. coli JM109/pEPI420 having been introduced therein with the mutant acid phosphatase gene and E. coli JM109/pEPI380 described in Example 19 was inoculated in 50 ml of an L medium containing 100 µg/ml of ampicillin and 1 mM of IPTG, and incubated at 37°C for 16 hours.

Pyrophosphoric acid (15 g/dl) and 8 g/dl of inosine or guanosine were dissolved in an acetate buffer (pH 4.0). To this was added E. coli JM109 strain having introduced therein each mutant acid phosphatase gene such that the concentration reached 100 mg/dl in terms of the dry cell weight. The reaction was conducted at 50°C for 9 hours while maintaining the pH at 4.0, and the amount of 5'-inosinic acid or 5'-guanylic acid formed was measured. The results are shown in Table 17. The nucleoside phosphate ester formed was only a nucleoside-5'-phosphate ester, and the production of a nucleoside-2'-phosphate ester and a nucleoside-3'-phosphate ester as by-products was not observed at all. The reaction was also conducted at 35°C for 12 hours using E. coli JM109/pEPI380 strain as a control. The results are also shown in Table 17.

As described in Example 21, the nucleoside-5'-phosphate ester was formed and accumulated efficiently with E. coli JM109/pEPI380. By contrast, when the reaction was conducted using E. coli JM109/pEPI410 and E. coli JM109/pEPI420 having been introduced therein with the new mutant acid phosphatase gene derived from E. blattae as produced in Example 26, 5'-inosinic acid or 5'-guanylic acid in the same amount was formed and accumulated for a shorter period of time. Thus, the nucleoside-5'-phosphate ester could be produced more efficiently. Especially when using E. coli JM109/pEPI420, not only was the reaction time shortened, but also were 5'-inosinic acid and 5'-guanylic acid accumulated in larger amounts, and quite a high productivity was shown.

Table 17

Strain	Reaction temperature (°C)	Reaction time (hr)	Amount of 5'-inosinc acid formed (g/dl)	Amount of 5'-guanylic acid formed (g/dl)
E. coli JM109/pEPI380	30	12	12.05	5.78
E. coli JM109/pEPI410	50	9	11.85	5.80
E. coli JM109/pEIP420	50	9	12.60	6.11

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Annex to th d cription

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) PROTEIN FRAGMENT TYPE: N-termianl
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Morganella morganii
(B) STRAIN: NCIMB 10466
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro Asp Leu Tyr Tyr
 1               5                  10                  15
Leu Lys Asn Glu
            20
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INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 750 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Morganella morganii
(B) STRAIN: NCIMB 10466
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..747
(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..60
(ix) FEATURE:
(A) NAME/KEY:mat_peptide
(B) LOCATION:61..747
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG AAG AAG AAT ATT ATC GCC GGT TGT CTG TTC TCA CTG TTT TCC CTT      48
Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser Leu Phe Ser Leu
-20                 -15                 -10                 -5
TCC GCG CTG GCC GCG ATC CCG GCG GGC AAC GAT GCC ACC ACC AAG CCG      96
Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
                    1               5                  10
GAT TTA TAT TAT CTG AAA AAT GAA CAG GCT ATC GAC AGC CTG AAA CTG     144
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Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu
                15                  20                  25
TTA CCG CCA CCG CCG GAA GTC GGC AGT ATT CAG TTT TTA AAT GAT CAG      192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
     30                  35                  40
GCA ATG TAT GAG AAA GGC CGT ATG CTG CGC AAT ACC GAG CGC GGA AAA      240
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
 45                  50                  55                  60

CAG GCA CAG GCA GAT GCT GAC CTG GCC GCA GGG GGT GTG GCA ACC GCA      288
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                 65                  70                  75
TTT TCA GGG GCA TTC GGC TAT CCG ATA ACC GAA AAA GAC TCT CCG GAG      336
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
            80                  85                  90
CTG TAT AAA CTG CTG ACC AAT ATG ATT GAG GAT GCC GGT GAT CTT GCC      384
Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        95                  100                 105
ACC CGC TCC GCC AAA GAA CAT TAC ATG CGC ATC CGG CCG TTT GCG TTT      432
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
    110                 115                 120
TAC GGC ACA GAA ACC TGT AAT ACC AAA GAT CAG AAA AAA CTC TCC ACC      480
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
125                 130                 135                 140
AAC GGA TCT TAC CCG TCA GGT CAT ACG TCT ATC GGC TGG GCA ACC GCA      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                145                 150                 155
CTG GTG CTG GCG GAA GTG AAC CCG GCA AAT CAG GAT GCG ATT CTG GAA      576
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu
            160                 165                 170
CGG GGT TAT CAG CTC GGA CAG AGC CGG GTG ATT TGC GGC TAT CAC TGG      624
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        175                 180                 185
CAG AGT GAT GTG GAT GCC GCG CGG ATT GTC GGT TCA GCC GCT GTC GCG      672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
    190                 195                 200
ACA TTA CAT TCC GAT CCG GCA TTT CAG GCG CAG TTA GCG AAA GCC AAA      720
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
205                 210                 215                 220
CAG GAA TTT GCA CAA AAA TCA CAG AAA TAA                             750
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                225                 229
```

INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Morganella morganii

(B) STRAIN: NCIMB 10466

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser Leu Phe Ser Leu
-20                 -15                 -10                  -5
Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
                 1               5                  10
Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu
         15                  20                  25
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
     30                  35                  40
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
 45                  50                  55                  60
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                 65                  70                  75
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
             80                  85                  90
Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
         95                  100                 105
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
    110                 115                 120
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
125                 130                 135                 140
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                145                 150                 155
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu
            160                 165                 170
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        175                 180                 185
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
    190                 195                 200
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
205                 210                 215                 220
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                225                 229
```

INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 229 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Morganella morganii
(B) STRAIN: NCIMB 10466

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro Asp Leu Tyr Tyr
 1               5                  10                  15
Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu Leu Pro Pro Pro
            20                  25                  30
Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln Ala Met Tyr Glu
        35                  40                  45
Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys Gln Ala Gln Ala
    50                  55                  60
Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala Phe Ser Gly Ala
65                  70                  75                  80
Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu Leu Tyr Lys Leu
                85                  90                  95
Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
            100                 105                 110
Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly Thr Glu
        115                 120                 125
Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr Asn Gly Ser Tyr
    130                 135                 140
Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
145                 150                 155                 160
Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu Arg Gly Tyr Gln
                165                 170                 175
Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
            180                 185                 190
Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala Thr Leu His Ser
        195                 200                 205
Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys Gln Glu Phe Ala
    210                 215                 220
Gln Lys Ser Gln Lys
225                 229
```

INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) PROTEIN FRAGMENT TYPE: N-termianl

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia blattae
(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu
 1               5                  10                  15
```

INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 750 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..747

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 1..54

(ix) FEATURE:

(A) NAME/KEY:mat_peptide

(B) LOCATION:55..747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG AAA AAA CGT GTT CTG GCA GTT TGT TTT GCC GCA TTG TTC TCT TCT      48
Met Lys Lys Arg Val Leu Ala Val Cys Phe Ala Ala Leu Phe Ser Ser
-18         -15                 -10                 -5
CAG GCC CTG GCG CTG GTC GCT ACC GGC AAC GAC ACT ACC ACG AAA CCG      96
Gln Ala Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro
        1               5                   10
GAT CTC TAC TAC CTC AAG AAC AGT GAA GCC ATT AAC AGC CTG GCG CTG     144
Asp Leu Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu
15                  20                  25                  30
TTG CCG CCA CCA CCG GCG GTG GGC TCC ATT GCG TTT CTC AAC GAT CAG     192
Leu Pro Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
                35                  40                  45
GCC ATG TAT GAA CAG GGG CGC CTG CTG CGC AAC ACC GAA CGC GGT AAG     240
Ala Met Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
            50                  55                  60
CTG GCG GCG GAA GAT GCA AAC CTG AGC AGT GGC GGG GTG GCG AAT GCT     288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala
        65                  70                  75
TTC TCC GGC GCG TTT GGT AGC CCG ATC ACC GAA AAA GAC GCC CCG GCG     336
Phe Ser Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala
    80                  85                  90
CTG CAT AAA TTA CTG ACC AAT ATG ATT GAG GAC GCC GGG GAT CTG GCG     384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
95                  100                 105                 110
ACC CGC AGC GCG AAA GAT CAC TAT ATG CGC ATT CGT CCG TTC GCG TTT     432
```

```
Thr Arg Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe
                115                 120                 125
TAT GGG GTC TCT ACC TGT AAT ACC ACC GAG CAG GAC AAA CTG TCC AAA      480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
            130                 135                 140
AAT GGC TCT TAT CCG TCC GGG CAT ACC TCT ATC GGC TGG GCT ACT GCG      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
        145                 150                 155

CTG GTG CTG GCA GAG ATC AAC CCT CAG CGC CAG AAC GAG ATC CTG AAA      576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
    160                 165                 170
CGC GGT TAT GAG CTG GGC CAG AGC CGG GTG ATT TGC GGC TAC CAC TGG      624
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
175                 180                 185                 190
CAG AGT GAT GTG GAT GCC GCG CGG GTA GTG GGA TCT GCC GTT GTG GCG      672
Gln Ser Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala
                195                 200                 205
ACC CTG CAT ACC AAC CCG GCG TTC CAG CAG CAG TTG CAG AAA GCG AAG      720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
            210                 215                 220
GCC GAA TTC GCC CAG CAT CAG AAG AAA TAA                             750
Ala Glu Phe Ala Gln His Gln Lys Lys
        225                 230
```

INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Lys Lys Arg Val Leu Ala Val Cys Phe Ala Ala Leu Phe Ser Ser
-18          -15                 -10                 -5
Gln Ala Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro
        1                   5                   10
Asp Leu Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu
15                  20                  25                  30
Leu Pro Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
                35                  40                  45
Ala Met Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
            50                  55                  60
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala
        65                  70                  75
```

```
Phe Ser Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala
    80                  85                  90
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
95                  100                 105                 110
Thr Arg Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe
                115                 120                 125

Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
            130                 135                 140
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
        145                 150                 155
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
    160                 165                 170
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
175                 180                 185                 190
Gln Ser Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala
                195                 200                 205
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
            210                 215                 220
Ala Glu Phe Ala Gln His Gln Lys Lys
        225                 230
```

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 231 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu
  1               5                  10                  15
Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu Leu Pro
            20                  25                  30
Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln Ala Met
        35                  40                  45
Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys Leu Ala
    50                  55                  60
Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala Phe Ser
65                  70                  75                  80
Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala Leu His
                85                  90                  95
Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg
            100                 105                 110
Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly
```

```
        115                 120                 125
Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys Asn Gly
    130                 135                 140
Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala Leu Val
145                 150                 155                 160
Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys Arg Gly
                165                 170                 175
Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser
            180                 185                 190
Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala Thr Leu
        195                 200                 205
His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys Ala Glu
    210                 215                 220
Phe Ala Gln His Gln Lys Lys
225                 230
```

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CCTCGAGGTC GACGGTATCG                                              20
```

INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
ATTCGCCACA TCGCCACTGC T                                            21
```

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
TAGCCCAGCC GGTAGAGGTA TG 22

INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TGCATCTGCC TGCGCCTGCT TAC 23

INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
AACGCGCCGT AGAAAGCATT 20

INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
GTCCTGGTCT TTGGTATTAC A 21

INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
CACATCGCCA GCGGCCAGGT CTGCAT 26

INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
GCATATAGTG TTCTTTCGCG C 21

INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
ATTACAGGTT TCGACCCCAT AA 22

INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
TGATGCATGT CCGGGCTGTC TTTTT 25

INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTGGATCCTG TGGCTATCAT CACCT 25

INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGGATCCGA CGCGATTTTA CCATA 25

INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 747 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Providencia stuartii
(B) STRAIN: ATCC 29851
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..744
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
ATG AAA AAA CTA TTA GCA GTA TTC TGC GCA GGG GCT TTT GTT TCA ACC      48
Met Lys Lys Leu Leu Ala Val Phe Cys Ala Gly Ala Phe Val Ser Thr
  1               5                  10                  15
AGT GTA TTT GCG GCG ATC CCT CCC GGC AAT GAT GTG ACA ACT AAA CCC      96
Ser Val Phe Ala Ala Ile Pro Pro Gly Asn Asp Val Thr Thr Lys Pro
            20                  25                  30
GAT CTT TAT TAT TTA AAA AAC TCA CAG GCT ATT GAT AGT TTA GCG TTA     144
Asp Leu Tyr Tyr Leu Lys Asn Ser Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
TTG CCG CCA CCA CCT GAA GTG GGC AGT ATC TTA TTT TTA AAC GAC CAA     192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Leu Phe Leu Asn Asp Gln
    50                  55                  60
GCG ATG TAT GAA AAA GGC CGT TTA TTG CGA AAT ACT GAG CGT GGA GAA     240
```

```
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Glu
 65                  70                  75                  80
CAA GCC GCT AAG GAT GCT GAT CTG GCT GCG GGC GGT GTT GCG AAC GCA      288
Gln Ala Ala Lys Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Asn Ala
                 85                  90                  95
TTT TCT GAA GCT TTT GGT TAT CCC ATT ACC GAA AAG GAT GCG CCT GAA      336
Phe Ser Glu Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ala Pro Glu
            100                 105                 110
ATT CAT AAA TTG CTG ACG AAT ATG ATT GAA GAT GCG GGG GAT TTA GCA      384
Ile His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
ACT CGC TCA GCC AAA GAG AAA TAC ATG CGC ATT CGT CCA TTT GCG TTC      432
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
TAC GGT GTT GCT ACC TGT AAC ACG AAA GAT CAG GAC AAA TTA TCT AAG      480
Tyr Gly Val Ala Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
AAT GGC TCT TAT CCT TCT GGA CAC ACC GCA ATT GGC TGG GCA TCT GCA      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ala Ile Gly Trp Ala Ser Ala
                165                 170                 175
CTC GTA TTG TCA GAA ATT AAC CCA GAA AAC CAA GAT AAA ATT TTA AAA      576
Leu Val Leu Ser Glu Ile Asn Pro Glu Asn Gln Asp Lys Ile Leu Lys
            180                 185                 190
CGT GGT TAT GAA CTT GGC CAA AGC CGA GTC ATC TGT GGT TAC CAT TGG      624
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
CAA AGT GAT GTT GAT GCA GCT CGT ATC GTT GCA TCG GGT GCG GTA GCA      672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Ala Ser Gly Ala Val Ala
    210                 215                 220
ACT TTA CAC TCC AAC CCT GAA TTC CAA AAA CAG TTA CAA AAA GCC AAA      720
Thr Leu His Ser Asn Pro Glu Phe Gln Lys Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
GAC GAA TTT GCT AAA CTG AAA AAA TAG                                  747
Asp Glu Phe Ala Lys Leu Lys Lys
                245
```

INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 248 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Providencia stuartii
- (B) STRAIN: ATCC 29851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Lys Lys Leu Leu Ala Val Phe Cys Ala Gly Ala Phe Val Ser Thr
```

```
  1               5                  10                  15
Ser Val Phe Ala Ala Ile Pro Pro Gly Asn Asp Val Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ser Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Leu Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Glu
65                  70                  75                  80
Gln Ala Ala Lys Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Glu Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ala Pro Glu
            100                 105                 110
Ile His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ala Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ala Ile Gly Trp Ala Ser Ala
                165                 170                 175
Leu Val Leu Ser Glu Ile Asn Pro Glu Asn Gln Asp Lys Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Ala Ser Gly Ala Val Ala
    210                 215                 220
Thr Leu His Ser Asn Pro Glu Phe Gln Lys Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
Asp Glu Phe Ala Lys Leu Lys Lys
                245
```

INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 744 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Enterobacter aerogenes
- (B) STRAIN: IFO 12010

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
ATG AAA AAG CGC GTT CTC GCC CTC TGC CTC GCC AGC CTG TTT TCC GTT        48
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15

AAC GCT TTC GCG CTG GTC CCT GCC GGC AAT GAT GCA ACC ACC AAA CCG        96
Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30

GAT CTC TAT TAT CTG AAA AAT GCA CAG GCC ATC GAT AGT CTG GCG CTG       144
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45

TTG CCG CCG CCG CCG GAA GTT GGC AGC ATC GCA TTT TTA AAC GAT CAG       192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60

GCG ATG TAT GAG AAA GGA CGG CTG TTG CGC AAT ACC GAA CGT GGC AAG       240
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80

CTG GCG GCT GAA GAT GCT AAC CTG AGC GCC GGC GGC GTC GCG AAT GCC       288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95

TTC TCC AGC GCT TTT GGT TCG CCC ATC ACC GAA AAA GAC GCG CCG CAG       336
Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln
            100                 105                 110

TTA CAT AAG CTG CTG ACA AAT ATG ATT GAG GAT GCC GGC GAT CTG GCC       384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125

ACC CGC AGC GCG AAA GAG AAA TAT ATG CGC ATT CGC CCG TTT GCG TTC       432
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140

TAC GGC GTT TCA ACC TGT AAC ACT ACC GAG CAG GAC AAG CTG TCG AAA       480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160

AAC GGA TCT TAC CCT TCC GGC CAT ACC TCT ATC GGT TGG GCA ACC GCG       528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175

CTG GTA CTG GCG GAG ATC AAT CCG CAG CGG CAA AAC GAA ATT CTC AAA       576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190

CGC GGC TAT GAA TTG GGC GAA AGC CGG GTT ATC TGC GGC TAT CAT TGG       624
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205

CAG AGC GAT GTC GAT GCG GCG CGG ATA GTC GGC TCG GCG GTG GTG GCG       672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220

ACC CTG CAT ACC AAC CCG GCC TTC CAA CAG CAG TTG CAG AAA GCA AAG       720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
```

```
GAT GAA TTC GCC AAA ACG CAG AAG TAA                                    747
Asp Glu Phe Ala Lys Thr Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 248 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Enterobacter aerogenes
- (B) STRAIN: IFO 12010

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
  1               5                  10                  15
Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln
            100                 105                 110
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
Asp Glu Phe Ala Lys Thr Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 747 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Klevsiella planticola
(B) STRAIN: IFO 14939

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATG AAA AAG CGT GTA CTC GCC CTT TGC CTT GCC AGC CTC TTT TCA GTT       48
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15
AGC GCC TTT GCG CTG GTT CCC GCC GGC AAT GAT GCC ACC ACC AAG CCC       96
Ser Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
GAT CTC TAC TAT CTG AAA AAT GCC CAG GCC ATT GAC AGC CTG GCG CTG      144
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45

TTG CCA CCG CCG CCG GAA GTG GGC AGC ATT GCG TTT TTA AAC GAT CAG      192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
GCG ATG TAT GAG AAA GGC CGT CTG CTG CGC GCC ACC GCC CGC GGC AAG      240
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Ala Thr Ala Arg Gly Lys
65                  70                  75                  80
TTG GCG GCA GAA GAT GCC AAC CTG AGC GCG GGT GGC GTG GCC AAC GCC      288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
TTC TCC GCA GCA TTC GGC TCC CCG ATC AGC GAA AAA GAC GCC CCG GCG      336
Phe Ser Ala Ala Phe Gly Ser Pro Ile Ser Glu Lys Asp Ala Pro Ala
            100                 105                 110
CTG CAC AAA CTG CTC ACC AAC ATG ATT GAA GAC GCG GGC GAT CTG GCG      384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
ACC CGA GGC GCG AAA GAG AAG TAT ATG CGT ATT CGT CCG TTT GCC TTC      432
Thr Arg Gly Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
TAC GGC GTG TCC ACC TGC AAT ACC ACC GAA CAG GAT AAG CTG TCG AAA      480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
```

```
145                 150                 155                 160
AAC GGC TCC TAC CCT TCC GGA CAC ACC TCT ATC GGC TGG GCG ACC GCC      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
CTG GTG CTG GCC GAA ATC AAC CCG CAG CGC CAG AAT GAG ATT CTC AAG      576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
CGC GGC TAT GAG CTC GGT GAA AGT CGG GTG ATC TGC GGT TAC CAC TGG      624
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
CAG AGC GAT GTT GAC GCC GCG CGG ATT GTC GGC TCG GCG GTG GTT GCA      672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
ACC CTG CAT ACC AAT CCG GCC TTC CAG CAG CAG CTG CAA AAA GCC AAA      720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
GAC GAG TTT GCG AAA CAG CAG AAA TAG                                  747
Asp Glu Phe Ala Lys Gln Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 248 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Klevsiella planticola
- (B) STRAIN: IFO 14939

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
  1               5                  10                  15
Ser Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Ala Thr Ala Arg Gly Lys
65                  70                  75                  80
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Ala Ala Phe Gly Ser Pro Ile Ser Glu Lys Asp Ala Pro Ala
            100                 105                 110
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Gly Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
```

```
    130                 135                 140
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240

Asp Glu Phe Ala Lys Gln Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 735 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Serratia ficaria
- (B) STRAIN: IAM 13540

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
ATG AAA AAA ATA TTA TTA GCC ACA TTA AGC TGC GCC GCG TTG ACG CAG      48
Met Lys Lys Ile Leu Leu Ala Thr Leu Ser Cys Ala Ala Leu Thr Gln
  1               5                  10                  15
TTT TCC TTT GCC GCC AAA GAT GTC ACT ACC CAC CCT GAG GTT TAT TTT      96
Phe Ser Phe Ala Ala Lys Asp Val Thr Thr His Pro Glu Val Tyr Phe
            20                  25                  30
CTG CAA GAA TCA CAG TCC ATC GAC AGC CTG GCA CTA TTG CCG CCG CCG     144
Leu Gln Glu Ser Gln Ser Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro
        35                  40                  45
CCG GCG ATG GAC AGC ATT GAT TTC CTG AAT GAC AAA GCG CAA TAC GAC     192
Pro Ala Met Asp Ser Ile Asp Phe Leu Asn Asp Lys Ala Gln Tyr Asp
    50                  55                  60
GCC GGG AAA ATA GTG CGC AAT ACT CCG CGT GGC AAG CAG GCT TAT GAT     240
Ala Gly Lys Ile Val Arg Asn Thr Pro Arg Gly Lys Gln Ala Tyr Asp
```

```
 65                  70                  75                  80
GAC GCC CAC GTT GCC GGG GAC GGC GTT GCC GCC GCA TTT TCC AAC GCC      288
Asp Ala His Val Ala Gly Asp Gly Val Ala Ala Ala Phe Ser Asn Ala
                 85                  90                  95
TTC GGC CTA GAA ATA GCC CAA CGG AAA ACG CCG GAG CTG TTT AAG CTG      336
Phe Gly Leu Glu Ile Ala Gln Arg Lys Thr Pro Glu Leu Phe Lys Leu
            100                 105                 110
GTG ATG AAA ATG CGT GAA GAC GCC GGC GAT TTG GCG ACC CGC AGC GCC      384
Val Met Lys Met Arg Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
        115                 120                 125
AAA AAT CAC TAT ATG CGC ATT CGC CCC TTT GCG TTT TAT AAC GAA GCG      432
Lys Asn His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Asn Glu Ala
    130                 135                 140
ACC TGC CGA CCG GAC GAA GAA AGC ACC CTG TCG AAG AAC GGT TCT TAC      480
Thr Cys Arg Pro Asp Glu Glu Ser Thr Leu Ser Lys Asn Gly Ser Tyr
145                 150                 155                 160
CCT TCC GGC CAT ACC ACC ATC GGC TGG GCG ACC GCG CTG GTG CTG GCT      528
Pro Ser Gly His Thr Thr Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
                165                 170                 175


GAA ATC AAC CCC GCC AGG CAG GGT GAA ATC CTG CAG CGC GGC TAT GAT      576
Glu Ile Asn Pro Ala Arg Gln Gly Glu Ile Leu Gln Arg Gly Tyr Asp
            180                 185                 190
ATG GGC CAA AGC CGG GTT ATC TGC GGT TAT CAC TGG CAA AGC GAC GTG      624
Met Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
        195                 200                 205
ACT GCG GCG CGC ATG GCG GCG TCG GCC ATG GTG GCG CGT TTG CAT GCC      672
Thr Ala Ala Arg Met Ala Ala Ser Ala Met Val Ala Arg Leu His Ala
    210                 215                 220
GAA CCC ACC TTC GCC GCC CAG CTG CAA AAG GCC AAA GAC GAA TTC AAC      720
Glu Pro Thr Phe Ala Ala Gln Leu Gln Lys Ala Lys Asp Glu Phe Asn
225                 230                 235                 240
GGC CTG AAA AAG TAA                                              735
Gly Leu Lys Lys
```

INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 244 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serratia ficaria

(B) STRAIN: IAM 13540

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Lys Lys Ile Leu Leu Ala Thr Leu Ser Cys Ala Ala Leu Thr Gln

```
  1               5                  10                  15
Phe Ser Phe Ala Ala Lys Asp Val Thr Thr His Pro Glu Val Tyr Phe
            20                  25                  30
Leu Gln Glu Ser Gln Ser Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro
        35                  40                  45
Pro Ala Met Asp Ser Ile Asp Phe Leu Asn Asp Lys Ala Gln Tyr Asp
    50                  55                  60
Ala Gly Lys Ile Val Arg Asn Thr Pro Arg Gly Lys Gln Ala Tyr Asp
65                  70                  75                  80
Asp Ala His Val Ala Gly Asp Gly Val Ala Ala Ala Phe Ser Asn Ala
                85                  90                  95
Phe Gly Leu Glu Ile Ala Gln Arg Lys Thr Pro Glu Leu Phe Lys Leu
            100                 105                 110
Val Met Lys Met Arg Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
        115                 120                 125
Lys Asn His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Asn Glu Ala
    130                 135                 140
Thr Cys Arg Pro Asp Glu Glu Ser Thr Leu Ser Lys Asn Gly Ser Tyr
145                 150                 155                 160
Pro Ser Gly His Thr Thr Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
                165                 170                 175
Glu Ile Asn Pro Ala Arg Gln Gly Glu Ile Leu Gln Arg Gly Tyr Asp
            180                 185                 190
Met Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
        195                 200                 205
Thr Ala Ala Arg Met Ala Ala Ser Ala Met Val Ala Arg Leu His Ala
    210                 215                 220
Glu Pro Thr Phe Ala Ala Gln Leu Gln Lys Ala Lys Asp Glu Phe Asn
225                 230                 235                 240
Gly Leu Lys Lys
```

INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCGGCGTCA CCAATCATAT T 21

INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other DNA..synthetic DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCCGGTAGAG GCATGCCCGG A 21

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Ajinomoto Co., Inc.
(B) STREET: No.15-1, Kyobashi 1-chome, Chuo-ku
(C) CITY: Tokyo
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): 104 Japan

(ii) TITLE OF INVENTION: Method for producing nucleoside-5'-phosphate ester

(iii) NUMBER OF SEQUENCES: 30

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 97309365.1

INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(v) PROTEIN FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Morganella morganii
(B) STRAIN: NCIMB 10466
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro Asp Leu Tyr Tyr
 1               5                  10                  15
Leu Lys Asn Glu
            20
```

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 750 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Morganella morganii

(B) STRAIN: NCIMB 10466

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..747

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 1..60

(ix) FEATURE:

(A) NAME/KEY:mat_peptide

(B) LOCATION:61..747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATG AAG AAG AAT ATT ATC GCC GGT TGT CTG TTC TCA CTG TTT TCC CTT      48
Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser Leu Phe Ser Leu
-20                 -15                 -10                 -5
TCC GCG CTG GCC GCG ATC CCG GCG GGC AAC GAT GCC ACC ACC AAG CCG      96
Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
                1                   5                  10
GAT TTA TAT TAT CTG AAA AAT GAA CAG GCT ATC GAC AGC CTG AAA CTG     144
Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu
            15                  20                  25
TTA CCG CCA CCG CCG GAA GTC GGC AGT ATT CAG TTT TTA AAT GAT CAG     192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
        30                  35                  40
GCA ATG TAT GAG AAA GGC CGT ATG CTG CGC AAT ACC GAG CGC GGA AAA     240
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
    45                  50                  55                  60
CAG GCA CAG GCA GAT GCT GAC CTG GCC GCA GGG GGT GTG GCA ACC GCA     288
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                65                  70                  75
TTT TCA GGG GCA TTC GGC TAT CCG ATA ACC GAA AAA GAC TCT CCG GAG     336
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
            80                  85                  90
```

```
CTG TAT AAA CTG CTG ACC AAT ATG ATT GAG GAT GCC GGT GAT CTT GCC      384
Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
          95                  100                 105
ACC CGC TCC GCC AAA GAA CAT TAC ATG CGC ATC CGG CCG TTT GCG TTT      432
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
     110                 115                 120
TAC GGC ACA GAA ACC TGT AAT ACC AAA GAT CAG AAA AAA CTC TCC ACC      480
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
125                 130                 135                 140
AAC GGA TCT TAC CCG TCA GGT CAT ACG TCT ATC GGC TGG GCA ACC GCA      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                145                 150                 155
CTG GTG CTG GCG GAA GTG AAC CCG GCA AAT CAG GAT GCG ATT CTG GAA      576
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu
            160                 165                 170
CGG GGT TAT CAG CTC GGA CAG AGC CGG GTG ATT TGC GGC TAT CAC TGG      624
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        175                 180                 185
CAG AGT GAT GTG GAT GCC GCG CGG ATT GTC GGT TCA GCC GCT GTC GCG      672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
    190                 195                 200
ACA TTA CAT TCC GAT CCG GCA TTT CAG GCG CAG TTA GCG AAA GCC AAA      720
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
205                 210                 215                 220
CAG GAA TTT GCA CAA AAA TCA CAG AAA TAA                             750
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                225                 229
```

INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Morganella morganii

(B) STRAIN: NCIMB 10466

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Lys Lys Asn Ile Ile Ala Gly Cys Leu Phe Ser Leu Phe Ser Leu
-20                 -15                 -10                 -5
Ser Ala Leu Ala Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
                1                   5                  10
Asp Leu Tyr Tyr Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu
          15                  20                  25
```

```
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln
     30                  35                  40
Ala Met Tyr Glu Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys
 45                  50                  55                  60
Gln Ala Gln Ala Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala
                 65                  70                  75
Phe Ser Gly Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu
             80                  85                  90
Leu Tyr Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
         95                  100                 105
Thr Arg Ser Ala Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe
    110                 115                 120
Tyr Gly Thr Glu Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr
125                 130                 135                 140
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                145                 150                 155
Leu Val Leu Ala Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu
            160                 165                 170
Arg Gly Tyr Gln Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        175                 180                 185
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala
    190                 195                 200
Thr Leu His Ser Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys
205                 210                 215                 220
Gln Glu Phe Ala Gln Lys Ser Gln Lys
                225                 229
```

INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 229 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Morganella morganii

(B) STRAIN: NCIMB 10466

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ala Ile Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro Asp Leu Tyr Tyr
  1               5                  10                  15
Leu Lys Asn Glu Gln Ala Ile Asp Ser Leu Lys Leu Leu Pro Pro Pro
             20                  25                  30
Pro Glu Val Gly Ser Ile Gln Phe Leu Asn Asp Gln Ala Met Tyr Glu
         35                  40                  45
Lys Gly Arg Met Leu Arg Asn Thr Glu Arg Gly Lys Gln Ala Gln Ala
     50                  55                  60
```

```
Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Thr Ala Phe Ser Gly Ala
 65                  70                  75                  80
Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ser Pro Glu Leu Tyr Lys Leu
                85                  90                  95
Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
            100                 105                 110
Lys Glu His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly Thr Glu
        115                 120                 125
Thr Cys Asn Thr Lys Asp Gln Lys Lys Leu Ser Thr Asn Gly Ser Tyr
    130                 135                 140
Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
145                 150                 155                 160
Glu Val Asn Pro Ala Asn Gln Asp Ala Ile Leu Glu Arg Gly Tyr Gln
                165                 170                 175
Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
            180                 185                 190
Asp Ala Ala Arg Ile Val Gly Ser Ala Ala Val Ala Thr Leu His Ser
        195                 200                 205
Asp Pro Ala Phe Gln Ala Gln Leu Ala Lys Ala Lys Gln Glu Phe Ala
    210                 215                 220
Gln Lys Ser Gln Lys
225                 229
```

INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) PROTEIN FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu
 1               5                  10                  15
```

INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 750 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..747

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 1..54

(ix) FEATURE:

(A) NAME/KEY:mat_peptide

(B) LOCATION:55..747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG AAA AAA CGT GTT CTG GCA GTT TGT TTT GCC GCA TTG TTC TCT TCT      48
Met Lys Lys Arg Val Leu Ala Val Cys Phe Ala Ala Leu Phe Ser Ser
-18         -15                 -10                 -5
CAG GCC CTG GCG CTG GTC GCT ACC GGC AAC GAC ACT ACC ACG AAA CCG      96
Gln Ala Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro
        1               5                   10
GAT CTC TAC TAC CTC AAG AAC AGT GAA GCC ATT AAC AGC CTG GCG CTG     144
Asp Leu Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu
15                  20                  25                  30
TTG CCG CCA CCA CCG GCG GTG GGC TCC ATT GCG TTT CTC AAC GAT CAG     192
Leu Pro Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
                35                  40                  45
GCC ATG TAT GAA CAG GGG CGC CTG CTG CGC AAC ACC GAA CGC GGT AAG     240
Ala Met Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
            50                  55                  60
CTG GCG GCG GAA GAT GCA AAC CTG AGC AGT GGC GGG GTG GCG AAT GCT     288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala
        65                  70                  75
TTC TCC GGC GCG TTT GGT AGC CCG ATC ACC GAA AAA GAC GCC CCG GCG     336
Phe Ser Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala
    80                  85                  90
CTG CAT AAA TTA CTG ACC AAT ATG ATT GAG GAC GCC GGG GAT CTG GCG     384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
95                  100                 105                 110
ACC CGC AGC GCG AAA GAT CAC TAT ATG CGC ATT CGT CCG TTC GCG TTT     432
Thr Arg Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe
                115                 120                 125
TAT GGG GTC TCT ACC TGT AAT ACC ACC GAG CAG GAC AAA CTG TCC AAA     480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
            130                 135                 140
```

```
AAT GGC TCT TAT CCG TCC GGG CAT ACC TCT ATC GGC TGG GCT ACT GCG      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Il  Gly Trp Ala Thr Ala
        145                 150                 155

CTG GTG CTG GCA GAG ATC AAC CCT CAG CGC CAG AAC GAG ATC CTG AAA      576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
    160                 165                 170

CGC GGT TAT GAG CTG GGC CAG AGC CGG GTG ATT TGC GGC TAC CAC TGG      624
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
175                 180                 185                 190

CAG AGT GAT GTG GAT GCC GCG CGG GTA GTG GGA TCT GCC GTT GTG GCG      672
Gln Ser Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala
                195                 200                 205

ACC CTG CAT ACC AAC CCG GCG TTC CAG CAG CAG TTG CAG AAA GCG AAG      720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
            210                 215                 220

GCC GAA TTC GCC CAG CAT CAG AAG AAA TAA                             750
Ala Glu Phe Ala Gln His Gln Lys Lys
        225                 230
```

INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Lys Lys Arg Val Leu Ala Val Cys Phe Ala Ala Leu Phe Ser Ser
-18         -15                 -10                 -5
Gln Ala Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro
        1                   5                   10
Asp Leu Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu
15                  20                  25                  30
Leu Pro Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
                35                  40                  45
Ala Met Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
            50                  55                  60
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala
          65                  70                  75
Phe Ser Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala
    80                  85                  90
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
95                  100                 105                 110
```

```
Thr Arg Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe
                115                 120                 125
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
            130                 135                 140
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
        145                 150                 155
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
    160                 165                 170
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
175                 180                 185                 190
Gln Ser Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala
                195                 200                 205
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
            210                 215                 220
Ala Glu Phe Ala Gln His Gln Lys Lys
        225                 230
```

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 231 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia blattae

(B) STRAIN: JCM 1650

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Leu Ala Leu Val Ala Thr Gly Asn Asp Thr Thr Thr Lys Pro Asp Leu
  1               5                  10                  15
Tyr Tyr Leu Lys Asn Ser Glu Ala Ile Asn Ser Leu Ala Leu Leu Pro
             20                  25                  30
Pro Pro Pro Ala Val Gly Ser Ile Ala Phe Leu Asn Asp Gln Ala Met
         35                  40                  45
Tyr Glu Gln Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys Leu Ala
     50                  55                  60
Ala Glu Asp Ala Asn Leu Ser Ser Gly Gly Val Ala Asn Ala Phe Ser
 65                  70                  75                  80
Gly Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Ala Leu His
                 85                  90                  95
Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala Thr Arg
            100                 105                 110
Ser Ala Lys Asp His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Gly
        115                 120                 125
Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys Asn Gly
    130                 135                 140
```

Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala Leu Val
145 150 155 160
Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys Arg Gly
165 170 175
Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser
180 185 190
Asp Val Asp Ala Ala Arg Val Val Gly Ser Ala Val Val Ala Thr Leu
195 200 205
His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys Ala Glu
210 215 220
Phe Ala Gln His Gln Lys Lys
225 230

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCTCGAGGTC GACGGTATCG 20

INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATTCGCCACA TCGCCACTGC T 21

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TAGCCCAGCC GGTAGAGGTA TG 22

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCATCTGCC TGCGCCTGCT TAC 23

INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE; nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AACGCGCCGT AGAAAGCATT 20

INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTCCTGGTCT TTGGTATTAC A 21

INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CACATCGCCA GCGGCCAGGT CTGCAT 26

INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCATATAGTG TTCTTTCGCG C 21

INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATTACAGGTT TCGACCCCAT AA 22

INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGATGCATGT CCGGGCTGTC TTTTT 25

INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTGGATCCTG TGGCTATCAT CACCT 25

INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTGGATCCGA CGCGATTTTA CCATA 25

INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 747 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Providencia stuartii

(B) STRAIN: ATCC 29851

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
ATG AAA AAA CTA TTA GCA GTA TTC TGC GCA GGG GCT TTT GTT TCA ACC      48
Met Lys Lys Leu Leu Ala Val Phe Cys Ala Gly Ala Phe Val Ser Thr
 1               5                  10                  15
AGT GTA TTT GCG GCG ATC CCT CCC GGC AAT GAT GTG ACA ACT AAA CCC      96
Ser Val Phe Ala Ala Ile Pro Pro Gly Asn Asp Val Thr Thr Lys Pro
            20                  25                  30
GAT CTT TAT TAT TTA AAA AAC TCA CAG GCT ATT GAT AGT TTA GCG TTA     144
Asp Leu Tyr Tyr Leu Lys Asn Ser Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
TTG CCG CCA CCA CCT GAA GTG GGC AGT ATC TTA TTT TTA AAC GAC CAA     192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Leu Phe Leu Asn Asp Gln
    50                  55                  60
GCG ATG TAT GAA AAA GGC CGT TTA TTG CGA AAT ACT GAG CGT GGA GAA     240
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Glu
65                  70                  75                  80
CAA GCC GCT AAG GAT GCT GAT CTG GCT GCG GGC GGT GTT GCG AAC GCA     288
Gln Ala Ala Lys Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
TTT TCT GAA GCT TTT GGT TAT CCC ATT ACC GAA AAG GAT GCG CCT GAA     336
Phe Ser Glu Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ala Pro Glu
            100                 105                 110
ATT CAT AAA TTG CTG ACG AAT ATG ATT GAA GAT GCG GGG GAT TTA GCA     384
Ile His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
ACT CGC TCA GCC AAA GAG AAA TAC ATG CGC ATT CGT CCA TTT GCG TTC     432
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
TAC GGT GTT GCT ACC TGT AAC ACG AAA GAT CAG GAC AAA TTA TCT AAG     480
Tyr Gly Val Ala Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
AAT GGC TCT TAT CCT TCT GGA CAC ACC GCA ATT GGC TGG GCA TCT GCA     528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ala Ile Gly Trp Ala Ser Ala
                165                 170                 175
CTC GTA TTG TCA GAA ATT AAC CCA GAA AAC CAA GAT AAA ATT TTA AAA     576
Leu Val Leu Ser Glu Ile Asn Pro Glu Asn Gln Asp Lys Ile Leu Lys
            180                 185                 190
CGT GGT TAT GAA CTT GGC CAA AGC CGA GTC ATC TGT GGT TAC CAT TGG     624
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
CAA AGT GAT GTT GAT GCA GCT CGT ATC GTT GCA TCG GGT GCG GTA GCA     672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Ala Ser Gly Ala Val Ala
    210                 215                 220
```

```
ACT TTA CAC TCC AAC CCT GAA TTC CAA AAA CAG TTA CAA AAA GCC AAA      720
Thr Leu His Ser Asn Pro Glu Phe Gln Lys Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
GAC GAA TTT GCT AAA CTG AAA AAA TAG                              747
Asp Glu Phe Ala Lys Leu Lys Lys
                245
```

INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Providencia stuartii

(B) STRAIN: ATCC 29851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Lys Lys Leu Leu Ala Val Phe Cys Ala Gly Ala Phe Val Ser Thr
 1               5                  10                  15
Ser Val Phe Ala Ala Ile Pro Pro Gly Asn Asp Val Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ser Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Leu Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Glu
65                  70                  75                  80
Gln Ala Ala Lys Asp Ala Asp Leu Ala Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Glu Ala Phe Gly Tyr Pro Ile Thr Glu Lys Asp Ala Pro Glu
            100                 105                 110
Ile His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ala Thr Cys Asn Thr Lys Asp Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ala Ile Gly Trp Ala Ser Ala
                165                 170                 175
Leu Val Leu Ser Glu Ile Asn Pro Glu Asn Gln Asp Lys Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Ala Ser Gly Ala Val Ala
    210                 215                 220
```

```
Thr Leu His Ser Asn Pro Glu Phe Gln Lys Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
Asp Glu Phe Ala Lys Leu Lys Lys
                245
```

INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 744 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Enterobacter aerogenes

(B) STRAIN: IFO 12010

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..744

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
ATG AAA AAG CGC GTT CTC GCC CTC TGC CTC GCC AGC CTG TTT TCC GTT      48
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15
AAC GCT TTC GCG CTG GTC CCT GCC GGC AAT GAT GCA ACC ACC AAA CCG      96
Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
GAT CTC TAT TAT CTG AAA AAT GCA CAG GCC ATC GAT AGT CTG GCG CTG     144
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
TTG CCG CCG CCG CCG GAA GTT GGC AGC ATC GCA TTT TTA AAC GAT CAG     192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
GCG ATG TAT GAG AAA GGA CGG CTG TTG CGC AAT ACC GAA CGT GGC AAG     240
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80
CTG GCG GCT GAA GAT GCT AAC CTG AGC GCC GGC GGC GTC GCG AAT GCC     288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
TTC TCC AGC GCT TTT GGT TCG CCC ATC ACC GAA AAA GAC GCG CCG CAG     336
Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln
            100                 105                 110
TTA CAT AAG CTG CTG ACA AAT ATG ATT GAG GAT GCC GGC GAT CTG GCC     384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
```

```
ACC CGC AGC GCG AAA GAG AAA TAT ATG CGC ATT CGC CCG TTT GCG TTC      432
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
TAC GGC GTT TCA ACC TGT AAC ACT ACC GAG CAG GAC AAG CTG TCG AAA      480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
AAC GGA TCT TAC CCT TCC GGC CAT ACC TCT ATC GGT TGG GCA ACC GCG      528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
CTG GTA CTG GCG GAG ATC AAT CCG CAG CGG CAA AAC GAA ATT CTC AAA      576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
CGC GGC TAT GAA TTG GGC GAA AGC CGG GTT ATC TGC GGC TAT CAT TGG      624
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
CAG AGC GAT GTC GAT GCG GCG CGG ATA GTC GGC TCG GCG GTG GTG GCG      672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
ACC CTG CAT ACC AAC CCG GCC TTC CAA CAG CAG TTG CAG AAA GCA AAG      720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
GAT GAA TTC GCC AAA ACG CAG AAG TAA                                  747
Asp Glu Phe Ala Lys Thr Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Enterobacter aerogenes

(B) STRAIN: IFO 12010

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15
Asn Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Asn Thr Glu Arg Gly Lys
65                  70                  75                  80
```

```
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Ser Ala Phe Gly Ser Pro Ile Thr Glu Lys Asp Ala Pro Gln
            100                 105                 110
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Ser Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
Asp Glu Phe Ala Lys Thr Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 747 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Klevsiella planticola

(B) STRAIN: IFO 14939

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATG AAA AAG CGT GTA CTC GCC CTT TGC CTT GCC AGC CTC TTT TCA GTT      48
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15
```

```
AGC GCC TTT GCG CTG GTT CCC GCC GGC AAT GAT GCC ACC ACC AAG CCC        96
Ser Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
                20                  25                  30
GAT CTC TAC TAT CTG AAA AAT GCC CAG GCC ATT GAC AGC CTG GCG CTG       144
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
            35                  40                  45
TTG CCA CCG CCG CCG GAA GTG GGC AGC ATT GCG TTT TTA AAC GAT CAG       192
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
        50                  55                  60
GCG ATG TAT GAG AAA GGC CGT CTG CTG CGC GCC ACC GCC CGC GGC AAG       240
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Ala Thr Ala Arg Gly Lys
65                  70                  75                  80
TTG GCG GCA GAA GAT GCC AAC CTG AGC GCG GGT GGC GTG GCC AAC GCC       288
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
TTC TCC GCA GCA TTC GGC TCC CCG ATC AGC GAA AAA GAC GCC CCG GCG       336
Phe Ser Ala Ala Phe Gly Ser Pro Ile Ser Glu Lys Asp Ala Pro Ala
            100                 105                 110
CTG CAC AAA CTG CTC ACC AAC ATG ATT GAA GAC GCG GGC GAT CTG GCG       384
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
ACC CGA GGC GCG AAA GAG AAG TAT ATG CGT ATT CGT CCG TTT GCC TTC       432
Thr Arg Gly Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
TAC GGC GTG TCC ACC TGC AAT ACC ACC GAA CAG GAT AAG CTG TCG AAA       480
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
AAC GGC TCC TAC CCT TCC GGA CAC ACC TCT ATC GGC TGG GCG ACC GCC       528
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
CTG GTG CTG GCC GAA ATC AAC CCG CAG CGC CAG AAT GAG ATT CTC AAG       576
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
CGC GGC TAT GAG CTC GGT GAA AGT CGG GTG ATC TGC GGT TAC CAC TGG       624
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
CAG AGC GAT GTT GAC GCC GCG CGG ATT GTC GGC TCG GCG GTG GTT GCA       672
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
ACC CTG CAT ACC AAT CCG GCC TTC CAG CAG CAG CTG CAA AAA GCC AAA       720
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240
GAC GAG TTT GCG AAA CAG CAG AAA TAG                                   747
Asp Glu Phe Ala Lys Gln Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Klevsiella planticola

(B) STRAIN: IFO 14939

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Lys Lys Arg Val Leu Ala Leu Cys Leu Ala Ser Leu Phe Ser Val
 1               5                  10                  15
Ser Ala Phe Ala Leu Val Pro Ala Gly Asn Asp Ala Thr Thr Lys Pro
            20                  25                  30
Asp Leu Tyr Tyr Leu Lys Asn Ala Gln Ala Ile Asp Ser Leu Ala Leu
        35                  40                  45
Leu Pro Pro Pro Pro Glu Val Gly Ser Ile Ala Phe Leu Asn Asp Gln
    50                  55                  60
Ala Met Tyr Glu Lys Gly Arg Leu Leu Arg Ala Thr Ala Arg Gly Lys
65                  70                  75                  80
Leu Ala Ala Glu Asp Ala Asn Leu Ser Ala Gly Gly Val Ala Asn Ala
                85                  90                  95
Phe Ser Ala Ala Phe Gly Ser Pro Ile Ser Glu Lys Asp Ala Pro Ala
            100                 105                 110
Leu His Lys Leu Leu Thr Asn Met Ile Glu Asp Ala Gly Asp Leu Ala
        115                 120                 125
Thr Arg Gly Ala Lys Glu Lys Tyr Met Arg Ile Arg Pro Phe Ala Phe
    130                 135                 140
Tyr Gly Val Ser Thr Cys Asn Thr Thr Glu Gln Asp Lys Leu Ser Lys
145                 150                 155                 160
Asn Gly Ser Tyr Pro Ser Gly His Thr Ser Ile Gly Trp Ala Thr Ala
                165                 170                 175
Leu Val Leu Ala Glu Ile Asn Pro Gln Arg Gln Asn Glu Ile Leu Lys
            180                 185                 190
Arg Gly Tyr Glu Leu Gly Glu Ser Arg Val Ile Cys Gly Tyr His Trp
        195                 200                 205
Gln Ser Asp Val Asp Ala Ala Arg Ile Val Gly Ser Ala Val Val Ala
    210                 215                 220
Thr Leu His Thr Asn Pro Ala Phe Gln Gln Gln Leu Gln Lys Ala Lys
225                 230                 235                 240

Asp Glu Phe Ala Lys Gln Gln Lys
                245
```

INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 735 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serratia ficaria

(B) STRAIN: IAM 13540

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
ATG AAA AAA ATA TTA TTA GCC ACA TTA AGC TGC GCC GCG TTG ACG CAG      48
Met Lys Lys Ile Leu Leu Ala Thr Leu Ser Cys Ala Ala Leu Thr Gln
 1               5                  10                  15
TTT TCC TTT GCC GCC AAA GAT GTC ACT ACC CAC CCT GAG GTT TAT TTT      96
Phe Ser Phe Ala Ala Lys Asp Val Thr Thr His Pro Glu Val Tyr Phe
            20                  25                  30
CTG CAA GAA TCA CAG TCC ATC GAC AGC CTG GCA CTA TTG CCG CCG CCG     144
Leu Gln Glu Ser Gln Ser Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro
        35                  40                  45
CCG GCG ATG GAC AGC ATT GAT TTC CTG AAT GAC AAA GCG CAA TAC GAC     192
Pro Ala Met Asp Ser Ile Asp Phe Leu Asn Asp Lys Ala Gln Tyr Asp
    50                  55                  60
GCC GGG AAA ATA GTG CGC AAT ACT CCG CGT GGC AAG CAG GCT TAT GAT     240
Ala Gly Lys Ile Val Arg Asn Thr Pro Arg Gly Lys Gln Ala Tyr Asp
65                  70                  75                  80
GAC GCC CAC GTT GCC GGG GAC GGC GTT GCC GCC GCA TTT TCC AAC GCC     288
Asp Ala His Val Ala Gly Asp Gly Val Ala Ala Ala Phe Ser Asn Ala
                85                  90                  95
TTC GGC CTA GAA ATA GCC CAA CGG AAA ACG CCG GAG CTG TTT AAG CTG     336
Phe Gly Leu Glu Ile Ala Gln Arg Lys Thr Pro Glu Leu Phe Lys Leu
            100                 105                 110
GTG ATG AAA ATG CGT GAA GAC GCC GGC GAT TTG GCG ACC CGC AGC GCC     384
Val Met Lys Met Arg Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
        115                 120                 125
AAA AAT CAC TAT ATG CGC ATT CGC CCC TTT GCG TTT TAT AAC GAA GCG     432
Lys Asn His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Asn Glu Ala
    130                 135                 140
```

```
ACC TGC CGA CCG GAC GAA GAA AGC ACC CTG TCG AAG AAC GGT TCT TAC      480
Thr Cys Arg Pro Asp Glu Glu Ser Thr Leu Ser Lys Asn Gly Ser Tyr
145                 150                 155                 160
CCT TCC GGC CAT ACC ACC ATC GGC TGG GCG ACC GCG CTG GTG CTG GCT      528
Pro Ser Gly His Thr Thr Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
                165                 170                 175
GAA ATC AAC CCC GCC AGG CAG GGT GAA ATC CTG CAG CGC GGC TAT GAT      576
Glu Ile Asn Pro Ala Arg Gln Gly Glu Ile Leu Gln Arg Gly Tyr Asp
            180                 185                 190
ATG GGC CAA AGC CGG GTT ATC TGC GGT TAT CAC TGG CAA AGC GAC GTG      624
Met Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
        195                 200                 205
ACT GCG GCG CGC ATG GCG GCG TCG GCC ATG GTG GCG CGT TTG CAT GCC      672
Thr Ala Ala Arg Met Ala Ala Ser Ala Met Val Ala Arg Leu His Ala
    210                 215                 220
GAA CCC ACC TTC GCC GCC CAG CTG CAA AAG GCC AAA GAC GAA TTC AAC      720
Glu Pro Thr Phe Ala Ala Gln Leu Gln Lys Ala Lys Asp Glu Phe Asn
225                 230                 235                 240
GGC CTG AAA AAG TAA                                                  735
Gly Leu Lys Lys
```

INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 244 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Serratia ficaria

(B) STRAIN: IAM 13540

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Lys Lys Ile Leu Leu Ala Thr Leu Ser Cys Ala Ala Leu Thr Gln
 1               5                  10                  15
Phe Ser Phe Ala Ala Lys Asp Val Thr Thr His Pro Glu Val Tyr Phe
            20                  25                  30
Leu Gln Glu Ser Gln Ser Ile Asp Ser Leu Ala Leu Leu Pro Pro Pro
        35                  40                  45
Pro Ala Met Asp Ser Ile Asp Phe Leu Asn Asp Lys Ala Gln Tyr Asp
    50                  55                  60
Ala Gly Lys Ile Val Arg Asn Thr Pro Arg Gly Lys Gln Ala Tyr Asp
65                  70                  75                  80
Asp Ala His Val Ala Gly Asp Gly Val Ala Ala Ala Phe Ser Asn Ala
                85                  90                  95
Phe Gly Leu Glu Ile Ala Gln Arg Lys Thr Pro Glu Leu Phe Lys Leu
            100                 105                 110
```

```
Val Met Lys Met Arg Glu Asp Ala Gly Asp Leu Ala Thr Arg Ser Ala
        115                 120                 125
Lys Asn His Tyr Met Arg Ile Arg Pro Phe Ala Phe Tyr Asn Glu Ala
    130                 135                 140
Thr Cys Arg Pro Asp Glu Glu Ser Thr Leu Ser Lys Asn Gly Ser Tyr
145                 150                 155                 160
Pro Ser Gly His Thr Thr Ile Gly Trp Ala Thr Ala Leu Val Leu Ala
                165                 170                 175
Glu Ile Asn Pro Ala Arg Gln Gly Glu Ile Leu Gln Arg Gly Tyr Asp
            180                 185                 190
Met Gly Gln Ser Arg Val Ile Cys Gly Tyr His Trp Gln Ser Asp Val
        195                 200                 205
Thr Ala Ala Arg Met Ala Ala Ser Ala Met Val Ala Arg Leu His Ala
    210                 215                 220
Glu Pro Thr Phe Ala Ala Gln Leu Gln Lys Ala Lys Asp Glu Phe Asn
225                 230                 235                 240
Gly Leu Lys Lys
```

INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCGGCGTCA CCAATCATAT T 21

INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other DNA..synthetic DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCCGGTAGAG GCATGCCCGG A 21

Claim

1. A method for producing nucleosid -5'-phosphate ester comprising the steps of allowing an acid phosphatase having an increased affinity for the nucleoside and/or an incr ased temperature stability to act under a condition of pH 3.0 to 5.5 on a nucleoside and a phosphate group donor to produce nucleoside-5'-phosphate ester, and collecting it.

2. A method for producing nucleoside-5'-phosphate ester comprising the steps of allowing a microorganism to act under a condition of pH 3.0 to 5.5 on a nucleoside and a phosphate group donor to produce nucleoside -5'-phosphate ester, and collecting it, wherein the microorgansm is transformed with a recombinant DNA comprising a gene encoding an acid phosphate having an increased affinity for the nucleoside and/or an increased temperature stability.

3. The method for producing nucleoside-5'-phosphate ester according to claims 1 or 2, wherein the Km value for the nucleoside is below 100.

4. The method for producing nucleoside-5'-phosphate ester according to claims 1 or 2, wherein the acid phosphatase is stable at 50°C.

5. The method for producing nucleoside-5'-phosphate ester according to claims 1 or 2, wherein the acid phosphate having an increased affinity for the nucleoside is derived from abacterium belonging to the genus Escherichia, the genus Morganella, the genus Providencia, the genus Enterobacter, the genus Klebsiella or the genus Serratia.

6. The method for producing nucleoside-5'-phosphate ester according to claim 5, wherein the acid phosphatase comprises an amino acid sequence which is substantially identical with an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 25, 27, 29 and 31 in Sequence Listing, and said acid phosphatase has a mutation which increases the affinity for the nucleoside and/or the temperature stability on an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 25, 27, 29 and 31 in Sequence Listing.

7. The method for producing nucleoside-5'-phosphate ester according to claim 5, wherein said mutation is selected from the group consisting of substitutions of the 63rd leucine residue, the 65th alanine residue, the 66th glutamic acid residue, the 69th aspartic acid residue, the 71st serine residue, the 72nd serine residue, the 74th glycine residue, the 85th serine residue, the 92nd alanine residue, the 94th alanine residue, the 104th glutamic acid residue, the 116th aspartic acid residue, the 130th serine residue, the 135th threonine residue, the 136th glutamic acid residue, the 151th threonine residue and/or the 153rd isoleucine residue with another amino acid in SEQ ID NO: 8 in Sequence Listing.

8. The method for producing nucleoside-5'-phosphate ester according to claims 1 or 2, wherein said phosphate group donor is selected from the group consisting of polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, acetylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof.

9. A mutant acid phosphatase comprising an amino acid sequence which is substantially identical with an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 25, 27, 29 and 31 in Sequence Listing, and said acid phosphatase has a mutation which increases the affinity for the nucleoside and/or the temperature stability on an amino acid sequence selected from the group consisting of sequences illustrated in SEQ ID NOs: 4, 8, 25, 27, 29 and 31 in Sequence Listing.

10. A mutant acid phosphatase according to claim 9, wherein said mutation is selected from the group consisting of substitutions of the 63rd leucine residue, the 65th alanine residue, the 66th glutamic acid residue, the 69th aspartic acid residue, the 71st serine residue, the 72nd serine residue, the 74th glycine residue, the 85th serine residue, the 92nd alanine residue, the 94th alanine residue, the 104th threonine residue, the 116th aspartic acid residue, the 130th serine residue, the 135th threonine residue, the 136th glutamic acid residue, the 151st threonine residue and/or the 153rd isoleucine residue with another amino acid in SEQ ID NO: 8 in Sequence Listing.

11. A gene coding for the acid phosphatase as defined in claim 9

12. A recombinant DNA comprising th g ne as defined in claim 11.

13. A microorganism harboring the r combinant DNA as defined in claim 12.

Fig 1

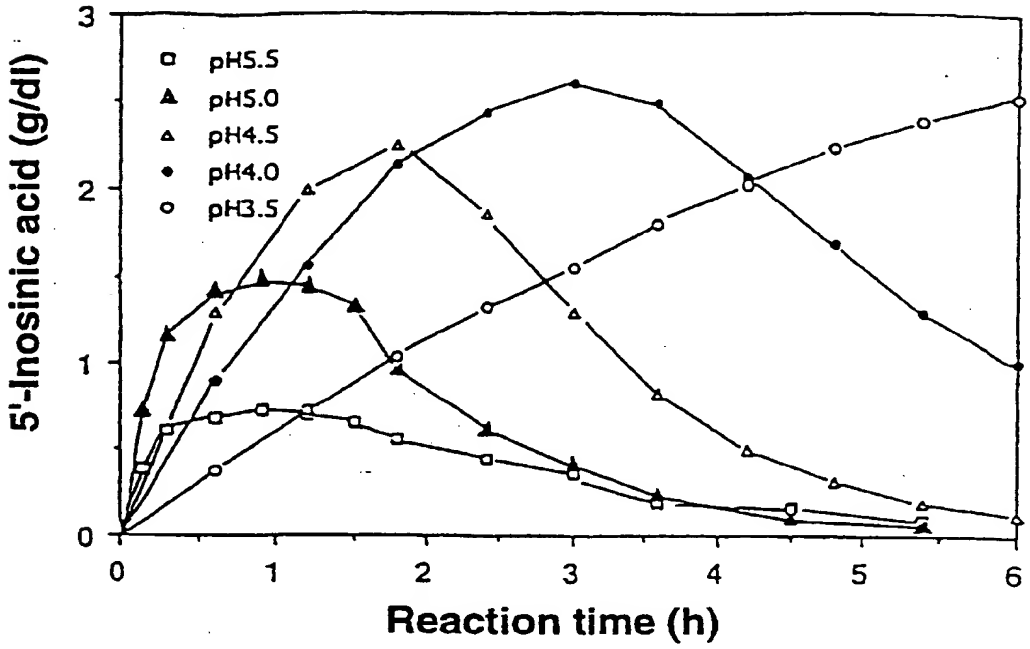

5'-Inosinic acid (g/dl)
Reaction time (h)
pH5.5
pH5.0
pH4.5
pH4.0
pH3.5
0
1
2
3
4
5
6

Fig 2

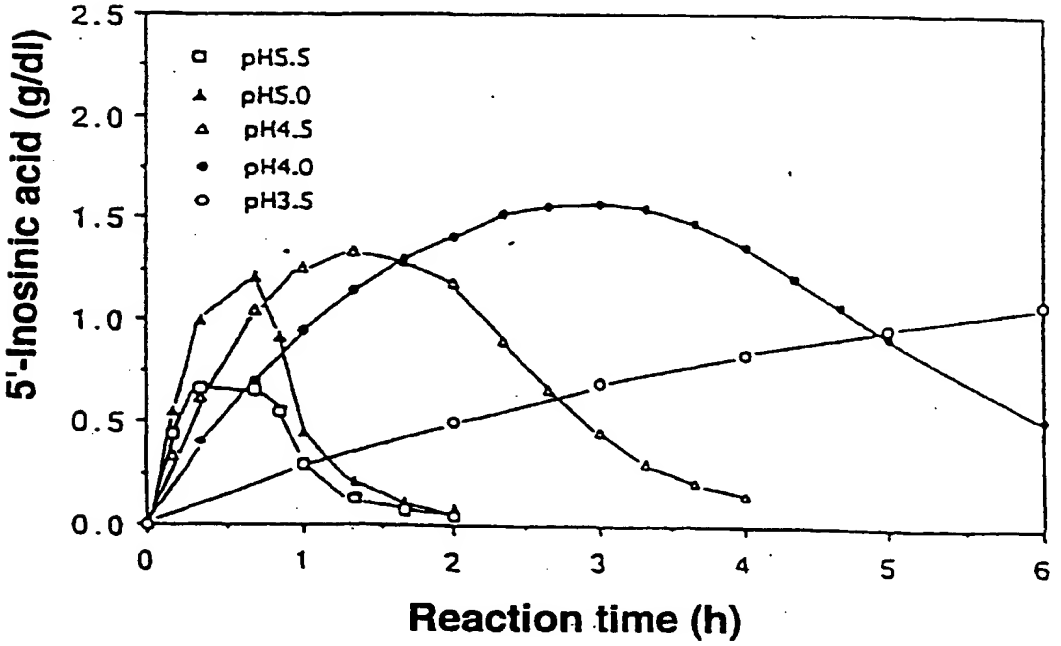
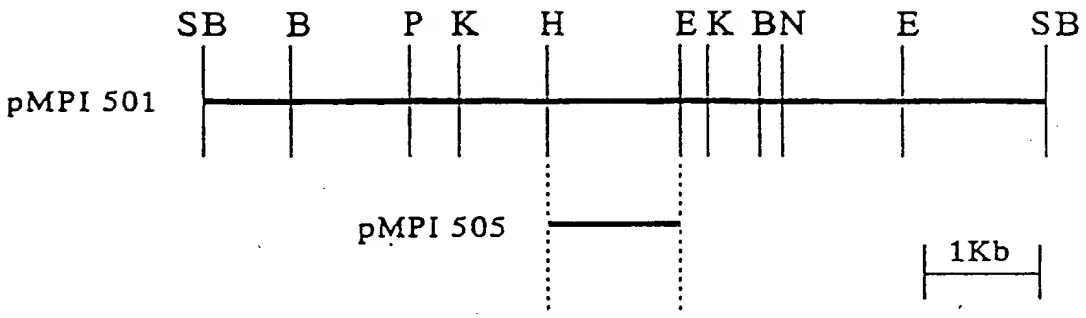
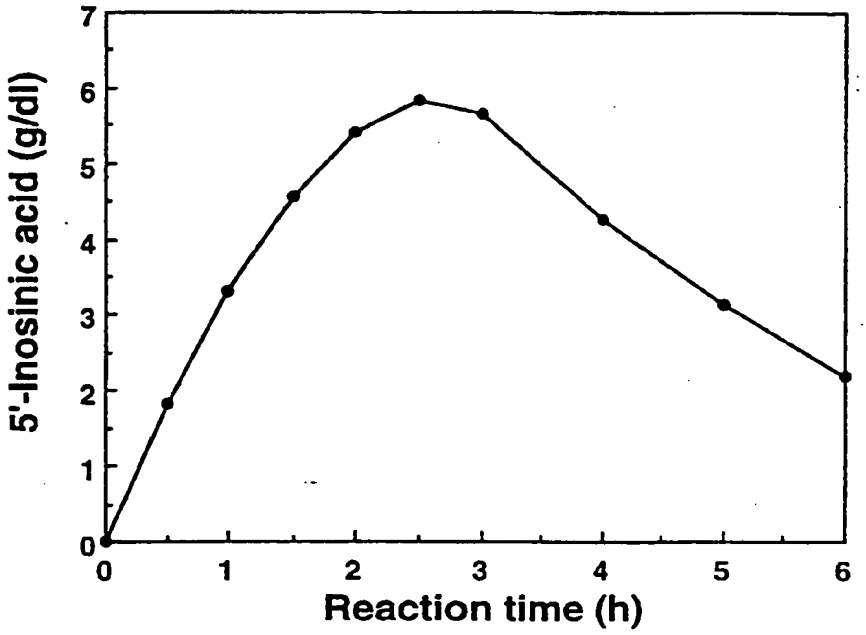

5'-Inosinic acid (g/dl)
Reaction time (h)
pH5.5
pH5.0
pH4.5
pH4.0
pH3.5
0.0
0.5
1.0
1.5
2.0
2.5
0
1
2
3
4
5
6

FIG. 3



SB: *Sau*3AI / *Bam*HI junction B: *Bam* HI E: *Eco*RI K: *Kpn*I
H: *Hin*dIII N: *Nco*I P: *Pst*I

【Fig.4】


5'-Inosinic acid (g/dl)
0
1
2
3
4
5
6
7
0
1
2
3
4
5
6
Reaction time (h)

【Fig.6】

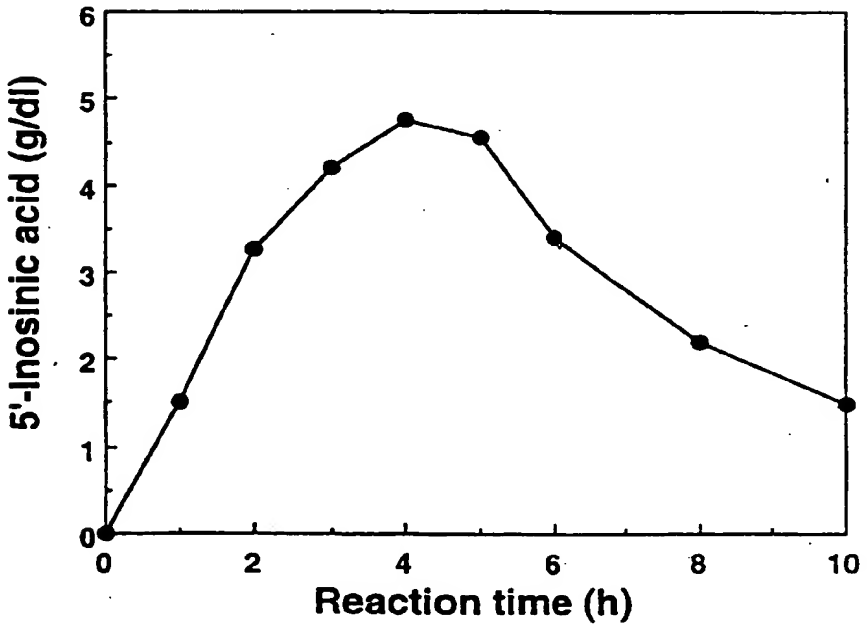
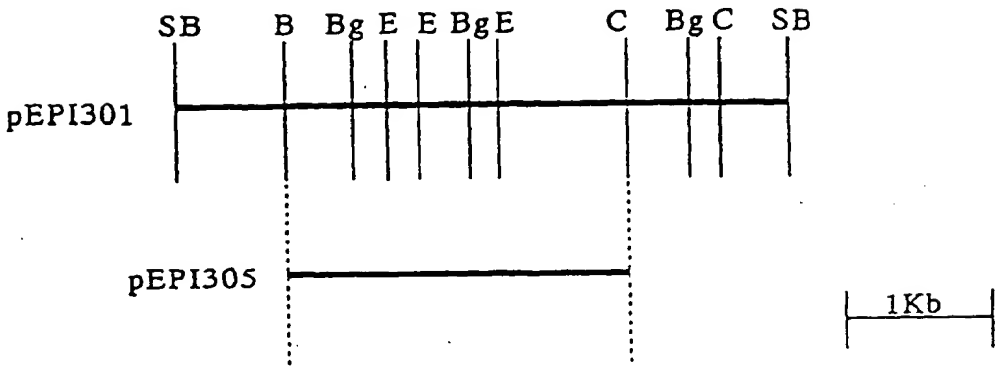

5'-Inosinic acid (g/dl)
0
1
2
3
4
5
6
0
2
4
6
8
10
Reaction time (h)

FIG. ~~6~~ 5



SB: *Sau*3AI / *Bam*HI junction B: *Bam*HI Bg: *Bgl*II C: *Cla*I E: *Eco*RI

【Fig.7】

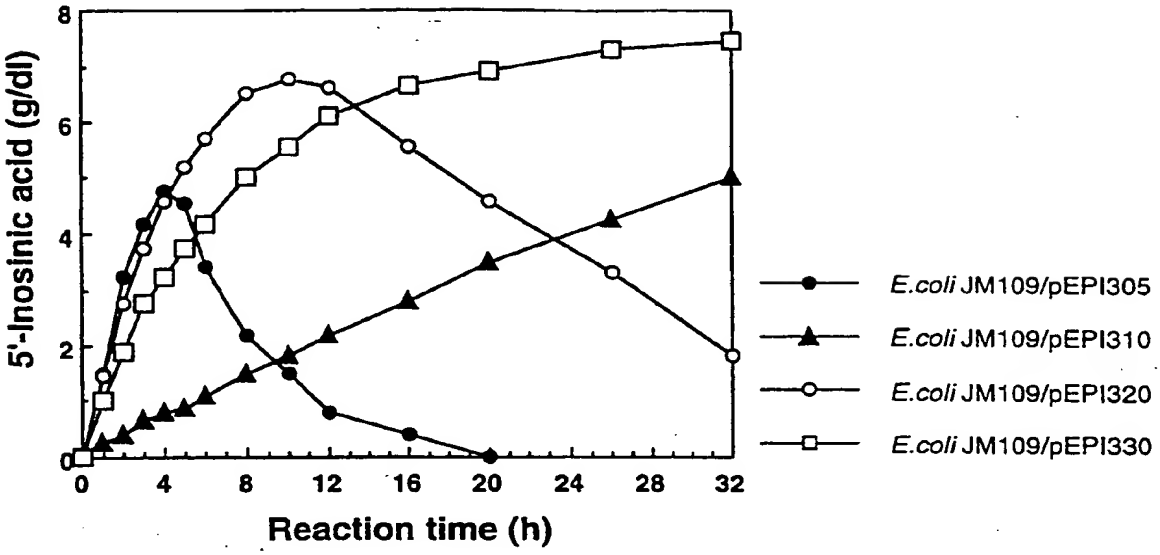

5'-Inosinic acid (g/dl)
0
2
4
6
8
0
4
8
12
16
20
24
28
32
Reaction time (h)
E.coli JM109/pEPI305
E.coli JM109/pEPI310
E.coli JM109/pEPI320
E.coli JM109/pEPI330

Fig 8

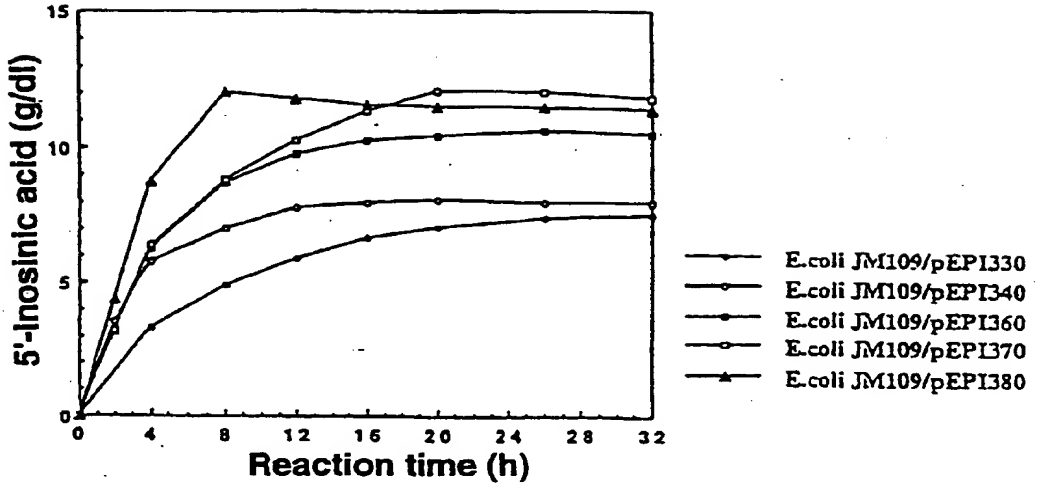

5'-Inosinic acid (g/dl)
Reaction time (h)
E.coli JM109/pEPI330
E.coli JM109/pEPI340
E.coli JM109/pEPI360
E.coli JM109/pEPI370
E.coli JM109/pEPI380

Fig 13

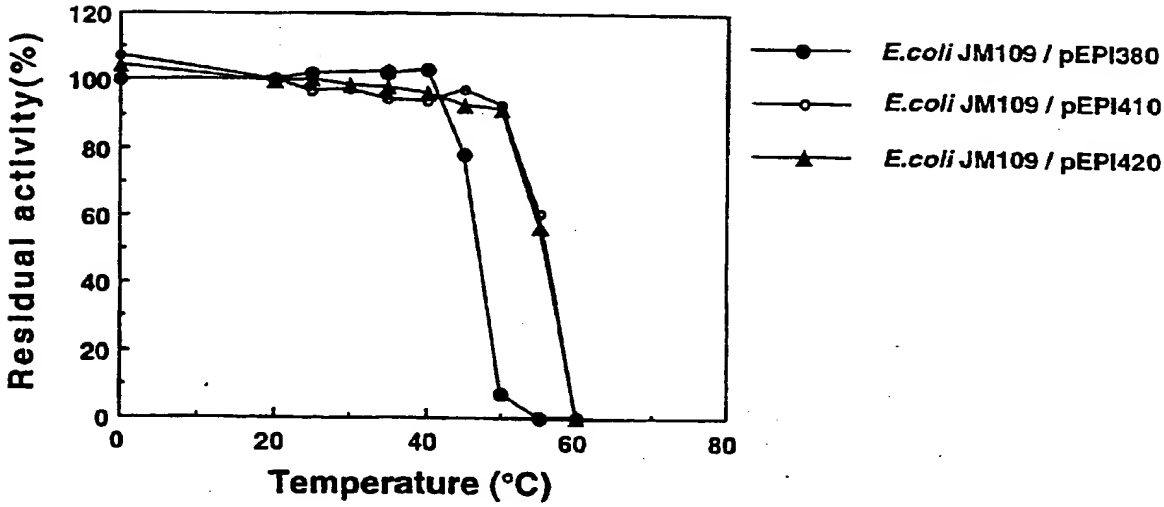
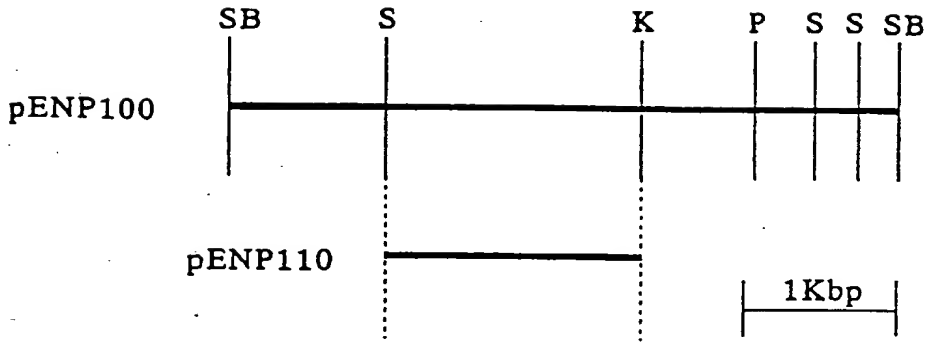
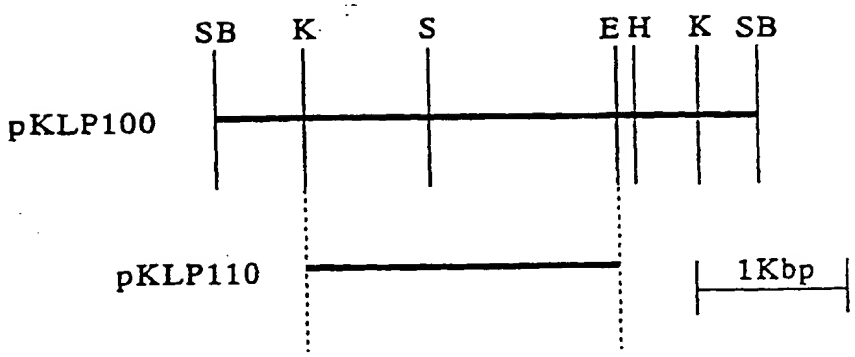

Residual activity(%)
Temperature (°C)
E.coli JM109 / pEPI380
E.coli JM109 / pEPI410
E.coli JM109 / pEPI420

FIG. 9



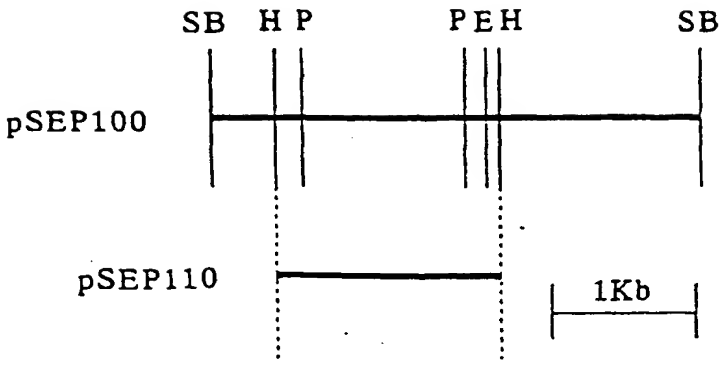
SB: *Sau*3AI / *Bam*HI junction K: *Kpn*I P: *Pst*I S: *Sal*I

FIG. 10



SB: *Sau*3AI / *Bam*HI junction E: *Eco*RI H: *Hin*dIII K: *Kpn*I
S: *Sac*I

FIG. 11



SB: *Sau*3AI / *Bam*HI junction E: *Eco*RI H: *Hin*dIII P: *Pst*I

FIG. 12

```
E.aerogenes      1:MKKRVLALCLASLFSVNAFALVPAGNDATTKPDLYYLKNAQAIDSLALLP  50
E.blattae        1:MKKRVLAVCFAALFSSQALALVATGNDTTTKPDLYYLKNSEAINSLALLP  50
K.planticola     1:MKKRVLALCLASLFSVSAFALVPAGNDATTKPDLYYLKNAQAIDSLALLP  50
M.morganii       1:MKKNIIAGCLFSLFSLSALAAIPAGNDATTKPDLYYLKNEQAIDSLKLLP  50
P.stuartii       1:MKKLLAVFCAGAFVSTSVFAAIPPGNDVTTKPDLYYLKNSQAIDSLALLP  50
S.ficaria        1:MKK-ILLA-TLSCAALTQFS--FAAKDVTTHPEVYFLQESQSIDSLALLP  46
                   ***                   * ** * * *    * ** ***

E.aerogenes     51:PPPEVGSIAFLNDQAMYEKGRLLRNTERGKLAAEDANLSAGGVANAFSSA 100
E.blattae       51:PPPAVGSIAFLNDQAMYEQGRLLRNTERGKLAAEDANLSSGGVANAFSGA 100
K.planticola    51:PPPEVGSIAFLNDQAMYEKGRLLRATARGKLAAEDANLSAGGVANAFSAA 100
M.morganii      51:PPPEVGSIQFLNDQAMYEKGRMLRNTERGKQAQADADLAAGGVATAFSGA 100
P.stuartii      51:PPPEVGSILFLNDQAMYEKGRLLRNTERGEQAAKDADLAAGGVANAFSEA 100
S.ficaria       47:PPPAMDSIDFLNDKAQYDAGKIVRNTPRGKQAYDDAHVAGDGVAAAFSNA  96
                   ***   ** **** * *  *   * * **  *  **  .   *** *** *

E.aerogenes    101:FGSPITEKDAPQLHKLLTNMIEDAGDLATRSAKEKYMRIRPFAFYGVSTC 150
E.blattae      101:FGSPITEKDAPALHKLLTNMIEDAGDLATRSAKDHYMRIRPFAFYGVSTC 150
K.planticola   101:FGSPISEKDAPALHKLLTNMIEDAGDLATRGAKEKYMRIRPFAFYGVSTC 150
M.morganii     101:FGYPITEKDSPELYKLLTNMIEDAGDLATRSAKEHYMRIRPFAFYGTETC 150
P.stuartii     101:FGYPITEKDAPEIHKLLTNMIEDAGDLATRSAKEKYMRIRPFAFYGVATC 150
S.ficaria       97:FGLEIAQRKTPELFKLVMKMREDAGDLATRSAKNHYMRIRPFAFYNEATC 146
                   **  *    *   **   * ********* **  **********   **

E.aerogenes    151:NTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELGES 200
E.blattae      151:NTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELGQS 200
K.planticola   151:NTTEQDKLSKNGSYPSGHTSIGWATALVLAEINPQRQNEILKRGYELGES 200
M.morganii     151:NTKDQKKLSTNGSYPSGHTSIGWATALVLAEVNPANQDAILERGYQLGQS 200
P.stuartii     151:NTKDQDKLSKNGSYPSGHTAIGWASALVLSEINPENQDKILKRGYELGQS 200
S.ficaria      147:RPDEESTLSKNGSYPSGHTTIGWATALVLAEINPARQGEILQRGYDMGQS 196
                     ** ********* **** **** * **  *  ** ***  * *

E.blattae      201:RVICGYHWQSDVDAARVVGSAVVATLHTNPAFQQQLQKAKAEFAQHQKK  249
K.planticola   201:RVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKQQK-  248
M.morganii     201:RVICGYHWQSDVDAARIVGSAAVATLHSDPAFQAQLAKAKQEFAQKSQK  249
E.aerogenes    201:RVICGYHWQSDVDAARIVGSAVVATLHTNPAFQQQLQKAKDEFAKTQK-  248
P.stuartii     201:RVICGYHWQSDVDAARIVASGAVATLHSNPEFQKQLQKAKDEFA-KLKK  248
S.ficaria      197:RVICGYHWQSDVTAARMAASAMVARLHAEPTFAAQLQKAKDEF-NGLKK  244
                   ************ ***   *  ** **  * *  ** *** **
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(19) Europäisches Patentamt
Europ an Patent Offic
Offic européen de br v ts

(11) EP 0 857 788 A3

(12) EUROPEAN PATENT APPLICATION

(88) Date of publication A3:
15.12.1999 Bulletin 1999/50

(43) Date of publication A2:
12.08.1998 Bulletin 1998/33

(21) Application number: **97309365.1**

(22) Date of filing: **20.11.1997**

(51) Int Cl.[6]: **C12N 15/55**, C12N 9/16, C12P 19/38

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: **21.11.1996 JP 31110396**
18.06.1997 JP 16167497

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(54) Method for producing nucleoside-5'-phosphate ester

(57) A method for producing nucleoside-5'-phosphate ester involves phosphorylating nucleoside biochemically.

Nucleoside-5'-phosphate ester is produced by allowing an acid phosphatase, especially an acid phosphatase having an increased affinity for a nucleoside and/or an increased temperature stability, to act at pH 3.0 to 5.5 on a nucleoside and a phosphate group donor. The phosphate group donor may be selected from polyphosphoric acid or a salt thereof, phenylphosphoric acid or a salt thereof, and carbamyl phosphate or a salt thereof. The resulting nucleoside-5'-phosphate ester may subsequently be collected. As an alternative to using isolated acid phosphatase to catalyse the reaction, a microorganism transformed with a gene encoding a protein having acid phosphatase activity may be employed.

EP 0 857 788 A3

Printed by Jouve, 75001 PARIS (FR)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 97 30 9365

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	MARIA CRISTINA THALLER: "Characterization and sequence of PhoC, the principal phosphate-irrepressible acid phosphatase of Morganella morganii" MICROBIOLOGY, vol. 140, no. 6, June 1994 (1994-06), pages 1341-1350, XP002119390 * the whole document *	1-13	C12N15/55 C12N9/16 C12P19/38
D,A	DATABASE WPI Section Ch, Week 199544 Derwent Publications Ltd., London, GB; Class B04, AN 1995-339304 XP002119391 & JP 07 231793 A (AJINOMOTO KK), 5 September 1995 (1995-09-05) * abstract *	1-13	
T	EP 0 832 970 A (AJINOMOTO CO., INC.) 1 April 1998 (1998-04-01) * page 2, line 35 - page 7, line 26; examples 1-18 * & WO 96 37603 A (AJINOMOTO CO., INC.) 28 November 1996 (1996-11-28)		TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C12P

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	19 October 1999	Montero Lopez, B

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

19-10-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 7231793 A	05-09-1995	NONE	
EP 832970 A	01-04-1998	JP 9037785 A	10-02-1997
		PL 323493 A	30-03-1998
		CA 2221774 A	28-11-1996
		CN 1191566 A	26-08-1998
		HU 9900808 A	28-07-1997
		WO 9637603 A	28-11-1996